

THE RÔLES OF NUCLEUS AND CYTOPLASM IN MELANIN ELABORATION

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ONE FIGURE

The more recent work on the formation of melanin seeks to derive this pigment from chromatin elements. In 1889 Mertsching attributed the formation of melanin to the breaking down of the cell and especially to the destruction of the nucleus. Jarisch ('92) makes the statement, "Das Oberhautpigment entwickelt sich aus einer Kernsubstanz, dem Chromatin, oder einem diesem chemisch oder wenigstens räumlich nahe stehenden Körper." Rössle ('04), in the discussion of pigment formation in melanosarcomas, recognizes five stages in the process, based on the appearance of the nucleus. He believes the melanin granules to be small particles of chromatin which are extruded from the nucleus, impoverishing the latter in the process.

Aurel von Szily ('11) has made a notable contribution to the theory of pigment formation from chromatic elements. He worked on the elaboration of melanin in developing eyes of a variety of vertebrates and in melanotic tumors of the human eye. According to his results, the melanin granules arise as colorless rod-like bodies (Pigmentträger) extruded from the nucleus, being derived directly from chromatic elements. These Pigmentträger are typical for species and locality of production. They also correspond exactly with the size and form of the melanin particles met with in that species and location. After being freed from the nucleus and wandering to a more or less peripheral position in the cell, the colorless Pigmentträger become colored probably by the action of cell ferments. The pigmentation of the Pigmentträger begins at one end of it and proceeds to the other. The origin of the Pigmentträger from the chromatin and their transition into the cell cytoplasm

may be followed step by step. The nucleus which gives rise to them may be either productive or degenerative. In the former case, no impoverishment of the nucleus takes place; in the latter, pigment formation is accompanied by marked degenerative changes in the nucleus.

In 1910 Harrison presented evidence of a new type upon the question of pigment formation. In his paper on nerve growth *in vitro*, he mentioned and figured certain cells which became pigmented during the life of the cultures. To quote, Harrison found that "the pigment first arose as a round mass of granules lying just to one side of the nucleus. This (mass) gradually increased in size and then the pigment granules became scattered through the cytoplasm" ('10, p. 812).

In 1912, while working on the reactions to light of embryonic connective tissue melanophores, material for a careful study of the actual elaboration of the melanin granules themselves was found in the developing connective tissue and epithelial cells of embryos of *Rana pipiens*. For this study, Harrison plasma cultures, living embryos and serial sections of carefully fixed embryos were used. The development of the melanin was followed in embryos varying in length from 3 to 10 mm.

Though the melanophoric cells found in the epidermis of older frog larvae are certainly mesodermal in origin, many ectodermal cells of young embryos elaborate this pigment within themselves. The pigment, however, after existing for a time in the cells, gradually disappears. That connective tissue cells elaborate melanin has long been an established fact. The mode of its development in these two types of cells is the same.

Plasma cultures. Small pieces of mesenchyme and epithelium from *Rana pipiens* embryos 3 to 4 mm. long were implanted in the plasma of frogs of varying species. Seventeen primary cultures were made. These lived in good condition over periods varying from three to forty days. In the case of the older cultures, the plasma was changed at frequent intervals. From these primary cultures, secondary were made, to the number of sixteen, by removing fragments of tissue and reimplanting in new plasma. In all, thirty-three cultures were studied.

At first (fig. 1, A) the cells were clear and translucent, without

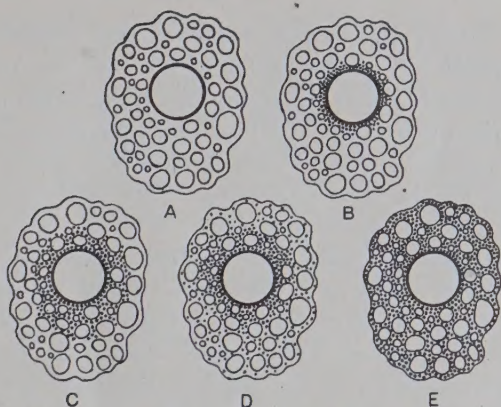


Fig. 1 Diagrammatic sketch to show the different stages of melanin elaboration. The heavily outlined circle in the center represents the nucleus of the cell, the smaller ovals represent oil droplets. The cell is represented in median section.

pigment granules, but contained fat droplets scattered throughout the cytoplasm and a more or less centrally placed nucleus. Then a few small, spherical, brownish granules became visible in the cytoplasm of the cell immediately adjacent to the nucleus, appearing simultaneously, or nearly so, on all sides of it. Their number gradually increased until a well defined hollow sphere surrounded the nucleus (fig. 1, B). On their first appearance, these melanin granules presented all the characteristics which are normal for them. No positive evidence of any increase in size of the individual particles of pigment was obtained. Owing, however, to their minuteness, an accurate determination of any such change presents almost insurmountable difficulties.

No granules made their appearance at any time inside the nuclear membrane nor in the cytoplasm of the cell away from the nucleus. The region of production was limited to that zone of the cytoplasm which is in contact with the nucleus. Nor was there any sign of the presence of colorless granules, which, by a process of pigmentation, could be transformed into the particles of melanin. The most careful search for any morphological structure within the cell that might serve as a *Pigmentträger* in von Szily's sense, was unavailing.

With each succeeding day, the number of melanin granules

increased. During the earlier stages of this process, they remained concentrated in the center of the cell, the periphery of the cloud of densely packed granules becoming larger and further away from the nucleus (fig. 1, C). Then the granules began to spread throughout the cell. This process was gradual and was accomplished by the slow separation of the individual elements of the dense cloud about the nucleus. At first, but a few granules, widely separated from one another, detached themselves from the central mass and wandered among the oil droplets nearest at hand. As this process of distribution, accompanied by further production of new granules around the nucleus, continued, more and more of the pigment particles spread toward the periphery (fig. 1, D), in such a manner that the number of granules per unit area steadily decreased from within outward. The periphery of the cell always contained fewer granules than the center until the final stage of melanin elaboration was reached (fig. 1, E). At this time, the cytoplasm of the cell was filled, one might say, to 'saturation.' Through this cloud of granules, the fat droplets and the nucleus were visible as clear, translucent areas, absolutely free from pigment. The entire process here described is completed in a period of eight to fourteen days, on an average.

Living embryos. By carefully dissecting out scraps of mesenchyme and epidermis from embryos of different ages and mounting them in plasma or isotonic saline (0.4 per cent), the different stages of melanin elaboration may be observed. That the steps thus seen are not continuous, but isolated from one another, is true. This objection to the method is obviated, however, by the check provided by the cultures. Every step in the elaboration of melanin observed in the cultures is exactly duplicated in the normal body. The light brown granules of melanin are found, at first, only in the region of the nucleus and then spread through the cytoplasm of the cell. They are colored on their first appearance, a fact which seems clearly to do away with the colorless Pigmentträger idea.

Serial sections. Like the study of fresh material from living embryos, that of serial sections serves principally as a check upon the cultures. A description of the findings here would

be but a repetition of facts already stated, with one important exception. The most minute examination of series fixed in all stages of melanin formation fails to show the slightest change in character and content or the least sign of degeneration or depletion in the nucleus. Nor can any evidence be found to show that the nucleus plays a part in the formation of melanin by a process of extrusion of any of its elements into the cytoplasm. All the granules of pigment are found in the cytoplasm near the nucleus, but they have no visible, structural connection with the nucleus or any of its contents.

Certain very important objections to the chromatin idea of the origin of melanin are evident from the results set forth above.

The nuclei of the pigment-forming cells suffer no depletion during the process. Though von Szily claims that certain nuclei which he terms 'active,' develop pigment without loss to their content, the actual depletion of many others was also seen by him. Rössle describes minutely many changes which occur in the nucleus and states that after the extrusion of chromatin to form melanin, the nucleus is bladderlike, with a reduced amount of chromatin.

No colorless anlagen for the melanin granules are to be found in the form of 'Pigmentträger' (v. Szily, '11) or 'Pigmentbildner' (Fischel, '96). That a chemical anlage in the form of a chromogen is present is almost certain in view of the work of Bertrand ('08) but that the melanogen exists in the frog as a definite morphological structure, which, without any other change than in its coloration, becomes the pigment granule itself, may be denied.

The process of pigmentation of a melanophore in the frog begins in the area nearest the nucleus and spreads from that point throughout the cell, that is to say, it progresses from the center of the cell to the periphery. This is in direct opposition to the observations of von Szily, who states that the pigmentation of the colorless Pigmentträger takes place gradually while they are wandering about in the cytoplasm. Indeed, his figure 4 (plate 4) shows the process going on irregularly throughout the cell and figure 8 of the same plate illustrates a process directly the reverse of that noted in this paper, namely, the appearance

of pigmented granules at the periphery of the cell before they are present near the nucleus.

The last, and probably the most important, objection to the supposed chromatin origin of melanin granules is the fact that no process of extrusion of chromatin nor any of the steps of such a process are to be observed. The pigment granules appear near the nucleus, in fact, in almost direct apposition to the nucleus, but no evidence was found in this work which even suggests a morphological relationship to either the nucleus or its contents.

It may safely be concluded that, in the normal ontogenetic origin of melanin in the frog, the chromatin plays no direct rôle. On the contrary, all the evidence obtained goes to demonstrate that the melanin granules are formed in the cytoplasm, from elements already present in solution in it, through some action of the nucleus.

Bertrand ('96) isolated an enzyme in plants (*Russula* and *Dahlia*) which, by its oxidizing action on tyrosin, was named tyrosinase. Von Fürth and Schneider ('01) found this same ferment in the haemolymph of Lepidopteran larvae and noted its occurrence in many animal forms. The action of this enzyme on tyrosin gives a melanin and von Fürth suggested,

. . . . dass die physiologische Bildung melaninartiger Pigmente in den tierischen Geweben auf das Zusammenwirken von zweierlei Fermenten zurückzuführen sei: durch ein autolytisches Ferment könnte ein aromatischer Komplex aus dem Eiweismaterial abgespalten und dieser sodann durch eine Tyrosinase in ein Melanin übergeführt werden. (1901, p. 242).¹

The remarkable results of Bertrand's ('08) more recent work demonstrate the manner in which tyrosin and its derivatives may form the various types of the melanins usually met with in the animal body. He determined that many substances may be transformed into melanin by the oxidizing action of tyrosinase, each giving a characteristic color. During oxidation, a play of colors results, the earlier stages of the process giving lighter colors than the more advanced. The essential constitu-

¹ It is not within the province of this paper to review in detail the literature on the melanins. The reader is referred to the excellent *Sammelreferat* of von Fürth ('04).

ent seems to be a benzene ring with an hydroxyl radicle. Tyrosin itself gives a black pigment, while paraoxyphenylacetic and paraoxyphenylpropionic acids give browns. It should be remembered, however, that the individual granules of 'black' melanin are brown; those of 'brown' melanins, yellowish in color.²

Gessard ('03) has given the strongest evidence yet adduced for the actual formation of melanin in the animal body from tyrosin. Working on melanotic tumors in horses, he determined the presence of free tyrosin and adds: "La tyrosine est donc le chromogène dont l'oxidation par la tyrosinase détermine la formation du pigment noir commun à divers produits physiologiques et pathologiques de l'économie animal." (p. 1088).

The recent work on protein digestion demonstrates that amino-acids are absorbed, unchanged, by the blood stream from the alimentary canal and are distributed to the tissues (Folin, '14). Van Slyke and Meyer ('13) have shown that "the disappearance of intravenously injected amino-acids from the circulation is the result of neither their destruction, synthesis nor chemical incorporation into cell proteins. The acids are merely absorbed from the blood by the tissues, without undergoing any immediate chemical change." They have also demonstrated that there is a limit to the amount of amino-acids that may be absorbed by the tissues, so that a certain equilibrium exists between the blood and the tissues so far as the amino-acids are concerned. Further, Osborne and Mendel ('12) have shown that, while certain amino-acid groups will sustain life if fed as an exclusive diet, "on the other hand it is clear that when certain amino-acid groups are lacking, nutritive equilibrium is impossible. The cyclic derivatives, *tyrosin* and tryptophane, appear to be included here" (p. 326).

Several of the protein putrefaction products mentioned by Bertrand as sources of melanin, as paracresol, paraoxyphenylacetic acid and others, all derivatives of tyrosin, also are known to be absorbed as such by the organism. There can be but little doubt that sufficient quantities of melanin-forming substances occur normally in the body.

² H. Eppinger ('10) isolated a melanogen from the urine of patients suffering from melanosisarcoma which turned black on oxidation. This he believes to be derived, not from a tyrosin base, but from tryptophane.

J. Loeb has repeatedly made the statement that oxidation is a prominent function of the nucleus in normal development and regeneration. Perhaps the most striking proof of this fact in specific tissues in the adult has been given by the work of R. S. Lillie ('02). By soaking thin slices of living tissues in solutions of substances which, colorless in the unoxidized condition, give brilliant color reactions on oxidation, he was able to determine the exact location in the individual cell where this reaction proceeds to the greatest extent. His findings "furnish, it is believed, conclusive evidence that in many tissues the nucleus is the chief agency in the intracellular activation of oxygen; and, further, that the active or atomic oxygen is in general most abundantly freed at the surface of contact between nucleus and cytoplasm" ('02, p. 420).

The findings in the normal development of melanin in the embryonic frog furnish strong histological evidence that the nucleus of the cells elaborating this pigment provides something vitally necessary for its production. The melanin granules appear, not in haphazard manner throughout the cell, but in the cytoplasm immediately about the nucleus or, in Lillie's words, "at the surface of contact between nucleus and cytoplasm." Lillie's work seems to indicate clearly that the vitally necessary element for melanin elaboration provided by the nucleus is an oxidizer. Jaquet in 1892 demonstrated that the oxidizing action of the cell was not alone a property of living tissue, but was also evinced by broken-down cells which were no longer living. Nevertheless, the oxidases present in dead cells were originally elaborated by the nucleus. Lillie's work demonstrates this.

The particular form which the oxidizing action of the nucleus takes in melanin elaboration is that of an oxidase, perhaps of a type of tyrosinase. A host of investigators, following in the footsteps of Bertrand's ('96) original discovery of the presence of tyrosinase in plants, have isolated this enzyme in many animal forms and in such bodily positions as to serve normally for the manufacture of melanin.

The data derived from these various sources may be briefly summed up as follows:

1. Tyrosin, or its derivatives, acted upon by an oxidizing

agent, tyrosinase, gives a melanin. (Bertrand, '96 and '08; von Fürth and Schneider, '01, etc.)

2. Free tyrosin was discovered by Gessard ('03) in horses with melanotic tumors and it is now a well known fact that derivatives of tyrosin are absorbed by the animal body.

3. Lillie ('02) gave definite proof of the rôle played by the nucleus as a producer of oxygen or of an oxidase.

4. The normal presence of tyrosinase discovered in many parts of the body by Gessard ('01, '02, '03,) Przibram ('01),³ Dewitz ('02), Durham ('04), Weindl ('07), etc.

When the histological data presented in this paper are considered in connection with the facts just reviewed, it will be seen that they are in full accord with one another. While no evidence has been obtained from this work that tyrosin is present in the cells under consideration, it is shown that the base from which the melanin granules are formed probably exists in a soluble condition in the cytoplasm. The rôle of the cytoplasm, then, is that of a carrier of the chromogen. That the nucleus plays an all important rôle is evident.

CONCLUSIONS

It is felt that the evidence here brought forward demonstrates conclusively the following points:

1. That the theory of the origin of melanin from chromatin elements extruded from the nucleus into the cytoplasm is untenable, at least in the frog.

2. That, however, the nucleus plays an essential part in pigment formation by some activity which greatly resembles an oxidizing action.

3. That melanin is formed in the cytoplasm of the cell at the point of known greatest efficiency of the nucleus as an oxidizing agent.

The following general conclusion from these facts seems justified: that, in the cells of embryo frogs, melanin is formed from some substance (probably tyrosin or its derivatives) in solution in the cytoplasm when acted upon by the nucleus (perhaps an oxidase reaction).

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³ Evidence given by von Fürth and Schneider ('01, p. 241).

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ON THE NORMAL SEX RATIO AND THE SIZE OF THE LITTER IN THE ALBINO RAT (*MUS* *NORVEGICUS ALBINUS*)

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ONE FIGURE

Literature dealing with the early development of the albino rat contains references to but two papers that give information regarding the normal sex ratio and litter size in this animal (Cuénot '99; King '11). Marked differences in the results of these two sets of investigations, which were made on relatively small numbers of individuals, render it necessary that a large series of observations should be recorded in order to furnish adequate standards by which one can judge the effects of experiments aiming to modify the sex ratio or to alter the fertility of the albino rat. To supply the material for such standards the data given in the present paper were collected.

All of the records given are of litters cast by stock albino rats kept in the animal colony of The Wistar Institute. During the period when the data were being collected (1911-1914) all of the animals used for breeding were subjected to similar environmental conditions, and they all were fed on a mixed diet that experience has shown is necessary if rats are to be kept in good condition for any length of time.

THE NORMAL SEX RATIO IN THE ALBINO RAT

Practically all of the data were obtained by examining litters at or very shortly after their birth, since the sexes can readily be distinguished at this time as Jackson ('12) has shown. The removal of the young rats from the nest entails some risk that the mother will not care for them after they are replaced, but it is necessary that the records be taken at this time if one wishes

an accurate determination of the sex ratio or of the litter size. Not infrequently litters contain one or more stillborn young which are usually eaten by the mother within a few hours after their birth. Often, too, some individuals in the litter, particularly if the litter is large, will be killed by the mother when they are several days old, or if one or more of the young rats in a large litter are constitutionally weak they will die from lack of nourishment, being unable to cope with their stronger brothers in their efforts to obtain food.

No attempt was made to obtain the sex records for all of the litters of stock albino rats that were born in the colony during the years 1911-1913. The data that were collected during this period have been grouped together, according to the months when the litters were cast, and are given in table 1.

TABLE 1

Showing the sex ratios and the average number of young in litters of stock albino rats born during 1911-1913. Data arranged according to the months when the litters were cast

MONTHS	NUMBER OF LITTERS	NUMBER OF INDIVID- UALS	MALES	FEMALES	NUMBER MALES TO 100 FEMALES	AVERAGE NO. YOUNG PER LITTER
January.....	28	194	103	91	113.2	6.9
February.....	18	123	65	58	112.1	6.8
March.....	32	236	113	123	92.2	7.3
April.....	16	101	47	54	87.0	6.3
May.....	21	135	69	66	104.5	6.4
June.....	27	194	108	86	125.6	7.1
July.....	22	160	87	73	119.2	7.2
August.....	12	77	40	37	108.1	6.4
September.....	11	80	38	42	90.5	7.2
October.....	46	316	159	157	101.3	6.8
November.....	16	117	65	52	125.0	7.3
December.....	26	195	102	93	109.7	7.5
	275	1928	996	932	106.9	7.01

One fact clearly brought out in the above table is that there is no restricted breeding season for the albino rat. Litters are cast during every month of the year, but, as the records for many thousands of litters show, relatively more litters are pro-

duced in the spring than during other seasons of the year. In table 1 the sex ratios for the different groups of litters do not show a very great range of variation considering the small number of litters involved. The highest sex ratio is that for the 27 litters cast during the month of June; the lowest sex ratio is found in the litters of the April group. For the entire series of 275 litters the sex ratio is 106.9 males to 100 females.

During the year 1914 an attempt was made to obtain the sex data for as many as possible of the litters of stock albino rats born in the colony. The cages containing the breeding animals were examined nearly every day throughout the year and practically all of the litters cast were recorded. The data obtained, arranged according to the months when the litters were cast, are given in table 2.

TABLE 2

Showing the sex ratios and the average number of young in litters of stock albino rats born during 1914. Data arranged according to the months when the litters were cast

MONTHS	NUMBER OF LITTERS	NUMBER OF INDIVID- UALS	MALES	FEMALES	NUMBER MALES TO 100 FEMALES	AVERAGE NO. YOUNG PER LITTER
January.....	57	407	202	205	98.5	7.1
February.....	56	410	197	213	92.5	7.3
March.....	58	432	210	222	94.6	7.4
April.....	51	367	199	168	118.4	7.1
May.....	60	430	217	213	101.9	7.1
June.....	101	744	387	357	108.4	7.3
July.....	116	821	430	391	109.9	7.0
August.....	109	776	438	338	129.6	7.1
September.....	111	751	377	374	100.8	6.7
October.....	31	187	104	83	125.3	6.0
November.....	33	188	99	89	111.2	5.7
December.....	31	178	96	82	117.1	5.7
	814	5691	2956	2735	108.1	6.99

Although the number of records taken during the year 1914 is about three times greater than that collected during the period 1911-1913, the range of variation in the sex ratios of the litters cast during the various months is only slightly greater than that given in table 1. The lowest sex ratio in this series

of records is found among the litters cast in February; the highest sex ratio occurs in the litters born in August. The sex ratio of the 814 litters examined during the entire year is 108.1 males to 100 females. This sex ratio is remarkably close to that found in the 275 litters previously recorded (table 1).

A summary for all of the data collected is given in table 3. In order to give equal value to the two sets of records the sex ratios in this table, and also the averages for the size of the litters cast in the various months, represent the arithmetical mean of the records as given in table 1 and in table 2; they have not been computed in any instance on a litter basis.

TABLE 3
A combination of the data given in table 1 and in table 2

MONTHS	NUMBER OF LITTERS	NUMBER OF INDIVID- UALS	MALES	FEMALES	NUMBER MALES TO 100 FEMALES	AVERAGE NO. YOUNG PER LITTER
January.....	85	601	305	296	105.7	7.0
February.....	74	533	262	271	102.3	7.0
March.....	90	668	323	345	93.4	7.3
April.....	67	468	246	222	102.7	6.7
May.....	81	565	286	279	103.2	6.7
June.....	128	938	495	443	116.9	7.2
July.....	138	981	517	464	114.6	7.1
August.....	121	853	478	375	118.8	6.7
September.....	122	831	415	416	95.6	6.9
October.....	77	503	263	240	113.3	6.4
November.....	49	305	164	141	118.1	6.5
December.....	57	373	198	175	113.4	6.6
	1089	7619	3952	3667	107.5	7.0

As arranged in table 3, the data show that the sex ratios are somewhat higher in the litters cast during the latter part of the year than in those cast in the early part of the year. With the exception of the record for March the sex ratios for the litter groups from January to May show a variation of less than three points; and the sex ratios for the litters cast from June to December, omitting the record for September, vary less than four points. The pronounced drop in the sex ratio for the litters produced during September is found in both sets of records, and at present there is no satisfactory explanation for it.

In the total of 1089 litters examined there were 3952 males and 3667 females, giving a sex ratio for the series of 107.5 males to 100 females. This sex ratio is somewhat higher than that given by Cuénot, who found in 30 litters of albino rats a sex ratio of 105.6 males to 100 females, but it is practically the same as that given by King ('11) for 80 litters of albino rats (107.3 males to 100 females). The sex ratio found among adult rats is doubtless considerably lower than that given above, as growth experiments with the albino rat at present under way seem to indicate that female rats, as a general thing, live longer than male rats and show somewhat less susceptibility to disease at all stages of their growth.

It would be futile to make a comparison between the sex ratios of the various litter groups owing to the inequality in the number of litters recorded for the different months. For the purpose of a somewhat closer analysis than that given above, the two sets of records have been grouped in table 4 according to the season of the year when the litters were cast. The averages given for the two sets of records were obtained in the same manner as were the averages in table 3.

TABLE 4

Showing the data for sex ratios and size of the litters in the albino rat arranged according to the season of the year when the litters were cast

SEASONS	1911-1913			1914			1911-1914	
	NUMBER OF LITTERS	NUMBER MALES TO 100 FEMALES	AVERAGE NO. YOUNG PER LITTER	NUMBER OF LITTERS	NUMBER MALES TO 100 FEMALES	AVERAGE NO. YOUNG PER LITTER	NUMBER MALES TO 100 FEMALES	AVERAGE NO. YOUNG PER LITTER
March to May....	69	94.2	6.8	169	103.8	7.2	99.0	7.0
June to August...	61	119.9	7.0	326	115.6	7.1	117.7	7.05
September to No- vember.....	73	104.4	7.0	175	106.2	6.4	105.3	6.7
December to Feb- ruary.....	72	111.6	7.1	144	99.0	6.9	105.3	7.0
	275	106.9	7.01	814	108.1	6.99	107.5	7.0

There is a very striking agreement between the corresponding sex ratios for the two sets of records, as is shown in table 4. In each case the sex ratio for the litters cast in the spring

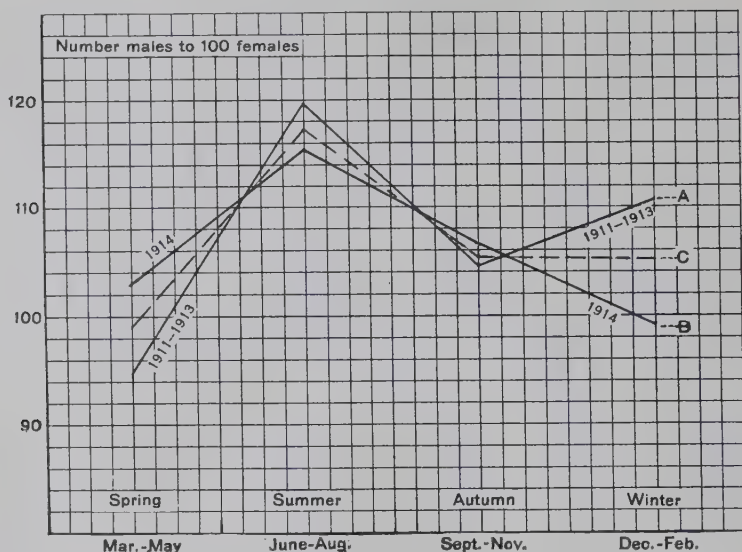


Fig. 1 Graphs showing variations in the sex ratio of the albino rat at different seasons of the year. A, graph constructed from data for litters cast during 1911-1913; B, graph constructed from data for litters cast during the year 1914; C, graph constructed from the averages for the two sets of data.

is considerably below the normal sex ratio of 107 males to 100 females; the average for the two groups giving a sex ratio of only 99.0 males to 100 females. Each set of data shows likewise a sharp rise in the sex ratios of the litters born during the summer months and then a drop to below the normal ratio for the litters born in the fall. The two sets of records for the litters cast in the winter months do not, for some reason, show the same agreement as those for the litters produced in other seasons of the year, as in one case the sex ratio is somewhat above the normal and in the other case it is below the normal.

Figure 1 shows graphs, constructed from the data given in table 4, which bring out very clearly the changes in the sex ratio that are found to occur among rats born at different seasons of the year.

Judging from observations and from the records for several thousand litters cast in our colony during the past six years,

the rat breeds more readily in the spring than in any other season of the year, and there is a second, less pronounced, period of sexual activity in the early fall. The lowest points in the graphs shown in figure 1 are found to coincide with the period in which the greatest sexual activity occurs. Lacking adequate means for heat regulation the rat suffers greatly from heat during the summer months, and for years the highest mortality among the animals in our colony has occurred in July and in August while relatively fewer litters are produced at this time than at other seasons of the year. It is during the hot weather when the breeding animals are not in the best physical condition that the litters produced show the highest sex ratio, as is indicated by the graphs in figure 1.

The seasonal variation in the sex ratios that is shown by these records cannot be ascribed to environmental conditions other than temperature, since the routine of caring for the animals in our colony is the same throughout the year and there is no change in the character of the food.

That the sex ratios in various mammals seem to show a pronounced variation at different seasons of the year has long been known. From the large body of statistics examined by Düsing ('83) it appears that relatively more boys are born during the winter than during the summer months. Table 5, compiled from data collected by Wilckens ('86) and by Heape ('08), shows the apparent seasonal variation in the sex ratio that occurs in the young of various kinds of domestic animals.

TABLE 5

Showing seasonal variations in the sex ratios of some domestic animals. Data collected by Wilckens and by Heape

ANIMALS	NUMBER INDIVIDUALS	NUMBER MALES TO 100 FEMALES		
		Birth in warm mos.	Birth in cold mos.	Birth during entire year
Horses.....	16,091	96.6	97.3	97.0
Cattle.....	4,900	114.1	103.0	107.3
Sheep.....	6,751	102.9	94.0	97.4
Swine.....	2,357	115.0	109.3	111.8
Greyhounds.....	17,838	116.3	122.1	118.5

Except in the dog, and in the horse where these statistics are at variance with those collected by Schlechter ('84), the sex ratios as given in table 5 are relatively high among the animals born in the warm months and correspondingly low where the births occurred during the cold months. To be available for analysis by any current theory of sex-determination, however, these records would have to be arranged according to the time when conception occurred, since it seems most probable that sex is determined at or before the time of the fertilization of the ovum and cannot be altered by the nutritive or other environmental conditions to which the embryo is subjected. The gestation period in the rat is so short, only 21 days, that the time of conception and the time of birth may be said to take place in the same season of the year. Since the gestation periods in the various animals for which sex ratios are given in table 5 vary so greatly, the sex records cannot be arranged on any basis except that of the time of birth, and they are of value, therefore, merely as indicating that there is apparently a seasonal variation in the sex ratio of other animals as well as in that of the albino rat.

If it can be shown by a sufficiently large body of statistics that the sex ratio in various animals changes in a definite direction with the time of year at which conception occurs it will indicate that some metabolic process occurs in one or the other or in both of the parent organisms at stated periods which tends to swing the sex ratio in one direction rather than in the other. Assuming that sex is determined by the chromatin constitution of the spermatozoan that fertilizes the egg, we must add to this theory the probability that some form of chemical attraction or repulsion exists between each ovum and one kind of spermatozoan in order to account for the constantly increasing mass of evidence that under changed environmental conditions sex ratios in various animals can be altered in a definite direction. Chance, therefore, cannot play as important a rôle in the process of sex-determination as some investigators have maintained, and *any* egg is not fertilized by *any* spermatozoan that happens to come in contact with it. The laws of chance, according to our

present conception, are not subject to periodic changes in their action, and while they offer a very attractive explanation for the existence of an equality of the sex in certain species, they utterly fail to explain sex ratios that vary in a definite direction, whether as the result of seasonal changes or as the outcome of experimental attempts to modify the sex ratio.

THE EFFECTS OF THE AGE OF THE MOTHER ON THE SEX RATIO OF HER YOUNG

It has been stated by many investigators that the age of the mother has a pronounced influence in determining the sex of her young. According to a considerable body of statistics collected by Punnett ('03) the sex ratio among the first children in a family is 140 boys to 100 girls. This ratio falls to 117 boys to 100 girls for the second births among the children of these same mothers, and it then declines steadily until, at the ninth birth, the chances for the two sexes are about even. In a compilation of birth statistics for the first born of women of various ages, Bidder ('78) found that the sex ratio was 122.2 boys to 100 girls when the mothers were under 19 years of age; this ratio falls to 104.6 boys to 100 girls for the children of women between 20-30 years of age and it then rises to 131 boys to 100 girls when the first conception occurs after the woman has reached 40 years of age. Conditions closely paralleling these for man are found in the horse according to Wilkens, but this investigator states that heifers predominate among the first offspring of cattle.

Data given by Copeman and Parsons ('04) from their in-breeding experiments with mice show the relation between the age of the mother and the sex of the offspring as given in table 6. Normally there is about an equal proportion of the sexes in mice as is shown by the investigations of Schultze ('03) and of Welden ('06).

The sex records for the mouse, as given in table 6, agree with those for man and for the horse in that they show that the sex ratio in the young is at its lowest point when the mother is at

the height of her reproductive powers. Schultze, on the other hand, states that young female mice tend to produce a slight excess of females among their young, and he concludes that the age of the mother has no effect whatever on the sex of her offspring.

TABLE 6

Showing the effects of the age of the mother on the sex ratio of mice. Data collected by Copeman and Parsons

AGE OF FEMALE AT CONCEPTION	NUMBER OF LITTERS	NUMBER OF MALES TO 100 FEMALES
2 mos.....	21	103.7
3-5 mos.....	27	96.5
6 mos.....	21	123.3

For comparison with the records given by Copeman and Parsons and by others we have the sex data for 75 litters cast by 21 stock albino rats. These data, arranged according to the location of the litter in the litter series, are given in table 7.

TABLE 7

Showing the sex ratios and average number of young in 75 litters of stock albino rats. Data arranged according to the position of the litters in the litter series

LITTER SERIES	NUMBER OF LITTERS	NUMBER OF INDIVIDUALS	MALES	FEMALES	NUMBER MALES TO 100 FEMALES	AVERAGE NO. YOUNG PER LITTER
1.....	21	131	72	59	122.0	6.2
2.....	21	162	85	77	110.4	7.7
3.....	18	127	64	63	101.6	7.0
4.....	15	96	41	55	74.5	6.4
	75	516	262	254	103.1	6.8

At the time that the first litter was cast each of the 21 females was about three months old. As shown in table 7, the sex ratio in the young rats belonging to the first litters is 122.0 males to 100 females. For the individuals in the second litters the sex ratio drops to 110.4 males to 100 females, and it goes down to 101.6 males to 100 females for the rats belonging to the third litters. At the time that the females cast their fourth litters the majority of them were seven to nine months old.

The female albino rat, if she is in good physical condition, will continue to bear young until she is about fifteen months old. The third and the fourth litters of an albino female, therefore, are usually cast during the period when the female is at the height of her reproductive power. In the above table the sex ratio for the fourth litters is much lower than that for the first three litters, being only 74.5 males to 100 females.

The records given in table 7 are, of course, too few to furnish evidence from which very definite conclusions can be drawn. As far as they go, however, these records indicate that the sex ratio among the first offspring of very young females is higher than that found among the offspring of the same females at a period of life when they are at the height of their reproductive power. The results, therefore, are in agreement with those obtained by Punnett, by Bidder and by Copeman and Parsons. In what way the age of the mother can affect the sex of her offspring is not known as yet. The fact that female rats at the height of their sexual activity in the spring and fall and also at the zenith of their reproductive power tend to produce relatively more female than male young would seem to indicate that the physical condition of the female, either as the result of age or of environment, produces changes of metabolism that tend to affect the sex of the young. It is possible that anabolic processes predominating in the female at certain periods might affect the ova in such a way as to cause them to be more easily fertilized by a female-producing than by a male-producing spermatozoan. In very young females, on the other hand, and in females not in good physical condition, katabolic processes that would give the male-producing spermatozoa an advantage over the female-producing spermatozoa in the fertilization of the ova, might be assumed to occur. Until, however, our knowledge of the mechanism of sex determination rests on a more secure foundation than it does at the present time, it seems useless to offer even tentative suggestions as to the manner in which this mechanism can be influenced.

THE RELATION BETWEEN THE SIZE OF A LITTER AND THE SEX OF ITS MEMBERS

Evidence for man as to whether one sex or the other tends to predominate in large families is conflicting. According to Nichols ('07), it has been shown by several investigators, particularly by Geissler ('89), that in large families there is a greater proportion of sons than in small families. Geissler's statistics show that in 159,042 families containing more than seven children the sex ratio was 106.8 boys to 100 girls, while in 839,719 families having from two to seven children each there were only 105.8 boys to 100 girls. From the statistics of a very much smaller number of families, Punnett ('03) comes to the opposite conclusion that girls tend to predominate more in large families than in small ones.

Copeman and Parsons's breeding experiments with mice show that the percentage of males is slightly less in large litters (containing more than 6 young) than it is in small litters. Welden, on the contrary, states that in a given generation of mice there seems to be a positive tendency for large litters to contain more males than females.

The sex data for 1089 litters of albino rats have been arranged on the basis of litter size in order to ascertain if, in this animal, there is any relation between the sex of the individuals and the size of the litters to which they belong. For the purpose of this analysis the litters have been arbitrarily divided into three groups: large litters containing nine or more young; medium litters with six to eight young; small litters having five or less members. The records collected during the year 1914 are sufficiently numerous to warrant their separation into groups according to the months when the litters were cast; the data obtained during 1911-1913, being too few to be divided in a similar way, have been grouped together. The results of this arrangement of data are given in table 8.

As shown in table 8, the results obtained by this analysis are so conflicting that no definite conclusions can be drawn from them. The data for the year 1914, arranged according to the months when the litters were cast, show that the highest sex

TABLE 8

Showing the sex ratio in different sized litters of albino rats. Data collected during 1914 arranged according to the months when the litters were cast

MONTHS	9 OR MORE YOUNG		6 TO 8 YOUNG		5 OR LESS YOUNG	
	Number litters	Number males to 100 females	Number litters	Number males to 100 females	Number litters	Number males to 100 females
January.....	14	108.7	27	93.1	16	91.7
February.....	19	98.9	25	87.6	12	87.5
March.....	24	87.1	22	98.7	12	125.0
April.....	14	112.3	24	144.3	13	75.8
May.....	23	104.6	19	85.9	18	126.5
June.....	34	100.6	42	109.9	25	131.3
July.....	36	97.7	56	120.6	24	116.7
August.....	29	129.9	57	134.1	23	111.4
September.....	28	100.0	46	98.1	37	107.8
October.....	1	125.0	18	119.3	12	140.9
November.....	3	93.8	15	123.4	15	100.0
December.....	3	130.8	15	106.3	13	133.3
	228	103.7	366	110.7	220	111.7
Data for 1911-1913.....	65	109.9	142	110.5	68	90.2
Total.....	293	106.8	508	110.6	288	100.7

ratio occurs in the members of the largest litters in only two cases, while in six cases it is found in the individuals comprising the smallest litters. In the records for the entire year the highest sex ratio, 111.7 males to 100 females, occurs in the individuals composing the smallest litters; the lowest sex ratio, 103.7 males to 100 females, being found in the rats belonging to the largest litters. The records for 1911-1913, on the other hand, give the highest sex ratio, 110.5 males to 100 females, in the individuals belonging to litters of medium size; the records for the small litters show a sex ratio of only 90.2 males to 100 females. For the entire series of data, litters of medium size show the highest sex ratio, 110.6 males to 100 females, and the lowest sex ratio occurs in the individuals of the small litters.

The lack of uniformity in the results of this arrangement of data indicate that apparently there is no well defined relation between litter size and sex in the albino rat.

THE NORMAL SIZE OF THE LITTER IN ALBINO RATS

Available data concerning litter size in the rat indicate that the average number of young in a litter varies considerably in different species. Miller ('11) finds for the common gray rat (*Mus norvegicus*) that there is a range of 7 to 12 young in the litter and that, on the average, a litter contains 10.5 young. Data recorded by Lantz ('10) give 8.1 as the average number of young in a large series of pregnant females of this species killed in India. Litters of the black rat (*Mus rattus*) are apparently much smaller than those of the gray rat. Lantz states that 5.2 young is the average for the litters of this species. This average is practically the same as that given by Lloyd ('09).

But few observations have been recorded regarding litter size in the albino rat. Crampe ('84) states that the average size of a litter of albino rats is 5.6 young, which is exactly the result obtained by one of us (King '11) from an examination of 80 litters of stock albino rats. Cuénot records 8.5 as the average number of young in 30 litters of albino rats, but this is undoubtedly a higher average than would be found in a larger series of litters.

In addition to the sex ratios tables 1-4 give the average number of young in the various litters examined during the years 1911-1914. The records for the period from 1911-1913, as given in table 1, show that there is very little variation in litter size in the various groups of litters cast during the different months of the year; the range being from 6.3 young, the average size of the litters cast during April, to 7.5 young, the average of the litters produced during December. The largest litter examined contained 14 young, the smallest contained only two individuals. For the series of 275 litters the average size of the litter was 7.01 young.

A similar analysis of the data collected during the year 1914, as given in table 2, shows for the entire series of 814 litters an average of 6.99 young per litter, which is remarkably close to the average for the litters examined in 1911-1913. While the records for 1914, as a whole, show a great uniformity in the

average size of the litters cast in the various months, there seems to be a tendency for the litters cast during the first part of the year to be slightly larger than those produced during the latter half of the year. A similar tendency, however, is not noted in the records of table 1, so that it can have little, if any, significance.

Records for the entire series of 1089 litters give 7.0 young as the average number of individuals in a litter. According to these observations, therefore, the size of a litter of albino rats is, on the average, greater than that of the black rat, but it is smaller than that in the gray rat of which it is the domesticated variety.

The data for litter size, arranged according to the season of the year when the litters were cast, are given in table 4. A marked uniformity in the various series of records is again evident. In the final averages the litters cast during the fall of the year show a relatively small size. This result probably has little, if any, meaning, since it is due entirely to the low average size of many of the litters cast during the fall of 1914. Records for the litters cast in corresponding months of the years 1911-1913 give 7.0 young as the average number of individuals per litter. It is evident, from these results, that there is no pronounced seasonal variation in the size of the litters at all comparable to the evident change that occurs in the sex ratio at stated periods in the year. Seasonal changes in the sex ratio are independent of litter size just as the normal sex ratio is independent of litter size.

Crampe ('83) states that the first litter of an albino rat is not as large as the second and that the second litter is an index of the size of subsequent litters. The first part of this statement can be corroborated by our records, but the latter part of it needs to be modified. A large second litter gives no indication whatever as to the size of the following litters, as the records for litters from many hundreds of females collected by one of us shows. In many cases a large second litter is followed by an unusually small litter, and there are marked individual differences in females regarding the size of the litters they pro-

duce. Some females never have over five or six young in a litter; other females invariably cast litters containing eight or more young.

The average size of 75 litters cast by 21 stock albino rats is given, with other data, in table 7. In these records the average size of the first litter is found to be considerably less than that of the second, while the second of the four litters is the largest of the group, containing an average of 7.7 young per litter. In this particular series of records the average size of the third litters is considerably below that for the second litters, but in a larger series of data it would probably be found that the third litter is nearly, if not equal, to the second in size. The fourth litters are, as shown in table 7, only a little larger than the first, as a rule.

For the entire series of 75 litters the sex ratio is below normal, and the average size of the litters is somewhat small, being only 6.8 young per litter. The number of young in a given litter is dependent to a marked extent on the age and physical condition of the female (King '15), and it is not improbable, as previously stated, that these factors also have an effect on metabolic processes that play an important rôle in determining the sex of the embryo.

SUMMARY

1. Albino rats breed throughout the entire year, but the periods of greatest sexual activity are in the spring and autumn.

2. The sex ratio in the 1089 litters of albino rats examined was 107.5 males to 100 females.

3. There is, apparently, a seasonal variation in the sex ratio of the albino rat. Litters cast in the spring and early fall show a relatively low sex ratio; those cast in summer have a much higher sex ratio (fig. 1).

4. Data for 75 litters produced by 21 albino females indicate that the sex ratio among the first offspring of young females is higher than that found among the offspring of the same females when they are at the height of their reproductive power.

5. There is apparently no relation between the size of a litter of albino rats and the sex of its members.

6. The 1089 litters examined contained an average of 7.0 young per litter. Litters of albino rats, therefore, are smaller than those of the gray rat and larger than the litters of the black rat.

7. There is no pronounced seasonal variation in the litter size comparable to the seasonal variation noted in the sex ratios.

8. As a rule the first of an albino female's four litters is the smallest; the second and the third litters are the largest; the fourth litter is a little larger than the first.

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AN INSTANCE OF ACIDOPHILIC CHROMOSOMES AND CHROMATIN PARTICLES

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ONE PLATE (TWELVE FIGURES)

Studying the cytological literature, I have been unable to find a record of acid staining chromosomes in a normally dividing cell. In an investigation of sections of petromyzon larvae numerous mesenchyma and blood cells are seen which contain nuclei staining a uniform and brilliant red. These cells are scattered among other cells having nuclei of exactly the same structure, yet staining the usual deep blue color with the hematoxilyn eosin stain. After studying these cells more closely, I found that the cells with the red nuclei were able to undergo mitotic division in the same manner as the cells with blue nuclei, the chromosomes in such cases staining red rather than the characteristic deep blue or black seen in the neighboring cells.

Such cells have been found as free blood cells in the blood vessels and also as mesenchyma cells in the pharyngeal and head regions of the larvae. Wherever found, aside from the peculiar property the nuclei show in the absorption of the acid dye, these cells are exactly similar to others in the region in which the nuclei stain characteristically with the basic dye (hematoxylin). The accompanying plate shows the two types of cells in the resting condition and in different stages of mitosis.

It is of interest that these cells have been found only in the 5 mm. larvae of my collection. These particular 5 mm. larvae were procured at Naples by Professor Stockard in the spring of 1910. They were fixed at the time of collection in picro-acetic and preserved in 80 per cent alcohol. I received them

in the fall of 1914, sectioned and stained them in hematoxylin and eosin. The remaining specimens of my collection, which comprise developmental stages ranging from the segmentation sphere up to an including a transforming larva and the adult, have been kindly supplied to me by Professor Gage. They were collected from the waters in the neighborhood of Ithaca, N. Y. Unfortunately, I do not have any 5 mm. larvae of the American species so am unable to say whether these cells are peculiar to the European species and to this age of larvae. Several specimens of these 5 mm. larvae show cells with nuclei taking the acid stain. They are not equally numerous nor do they take the stain equally well in all cases. In one specimen, for instance, such cells are difficult to find while in others they are distinct and plentiful. Embryos in which these cells are prominent may show more than half of the blood cells containing nuclei in which the chromatin has absorbed the acid dye.

Cells have been found in which both kinds of chromatin are present. Figure 7 represents such a cell in the resting state. Two lumps of chromatin in the central part of the nucleus have taken the basic stain while the chromatin at the periphery is stained with the acid dye. Heidenhain ('07) has shown a chromatolytic nucleus which somewhat resembles this, but in which the acid-staining chromatin was in the central part of the nucleus while the basic-staining chromatin was at the periphery. Figures 5 and 6 represent cells with two kinds of chromatin in the process of mitosis. Figure 6 shows that a small part of the acid-staining chromatin has apparently been taken over with the basic-staining group. Figure 5 represents the separation of the two kinds of chromosomes in the daughter nuclei which appears to have been complete. The great majority of these cells with acid-staining chromatin, however, are pure in regard to their staining reaction. Figures 1, 4, 8, 9 and 10 show nuclei containing no granule or any other part which absorbs the basic dye.

Stockard ('06) confirmed the observations of Schniewind-Theis ('97) in which it was shown that some nuclei in the deeper layers of actively secreting nectar glands of *Vicia faba* take the plasma

stain. In the living gland some rows of cells have a blue while others have a red coloration. By introducing alkaline and acid fluids to sections of the living gland, Stockard found that these cells responded to the fluids in the same way that litmus does to alkalies and acids. This experiment shows quite conclusively that the chemical reaction of the glandular plant cell is not constant during various physiological phases. The staining reaction also indicates that the nuclei apparently respond to the stain according to their physiological state. The stain used was Auerbach's (methyl green and acid fuchsin) which gives a delicate differentiation of the acid and basic qualities. It was determined in these investigations that materials were formed by the nucleus and passed out into the cytoplasm, the cytoplasm in such cases finally accumulating enough of the nuclear products to stain with the nuclear dye. Further, when the secreting activities of the nucleus had apparently been spent the nucleus stained with the plasma stain. These reactions occurred only in vegetative cells, the dividing cell always contained chromatin which stained in the normal way with the basic dye. In these studies the tissues had been carefully fixed with neutral fluids so as to preserve the chemical reaction of the living cell. The fixation used in my specimens, as was pointed out above, was an acid fluid, yet the differences in the reactions of the cells and portions of some nuclei were sufficient to maintain their character and to respond to the ordinary hematoxylin-eosin stain in the peculiar ways shown in plate 1.

The presence in the same section of the lamprey larva of cells with acidophilic nuclei together with cells which stain in the normal way, and the fact that both kinds of chromatin are present in a single cell, make it difficult to give any other interpretation of these reactions than that they represent the result of physiological changes which occurred during life.

As far as I can ascertain this is the first account of a case where the chromosomes in a dividing cell have definitely taken the acid stain.

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PLATE 1

EXPLANATION OF FIGURES

All figures were drawn with the aid of the camera lucida to the same scale of magnification (1/12 oil immersion objective, compensating ocular No.12). Higgins' carmine and true blue inks were used in reproducing the colors of the stained specimens.

Figures 1, 2 and 5 are mesenchyma cells; all other figures represent blood cells.



THE CONNECTING SYSTEMS OF THE REPTILE HEART

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EIGHT FIGURES (TWO PLATES)

THE SINO-VENTRICULAR CONNECTION

A sino-ventricular bundle, or dorsal ligament, has already been described by me in the hearts of *Lacerta agilis*, and *L. viridis*, of *Clemmys lutaria* and *Chelopus insculptus*. By observing this ligament in living hearts under the binocular microscope, and from the study of transverse, frontal and sagittal sections, it was seen to be a band of connective tissue, containing nerves and blood vessels, running between the sinus and the ventricle well over to the right side of the heart, (Laurens '13 a and '13 b). In my first paper it was further shown that this ligament had no significance for the coordination of the heart beat. This band of tissue had previously been described and experimented with by several investigators, and a discussion of their various views as to its structure and physiological importance will be found in my papers and in a recent publication by Mangold ('14).

Mackenzie ('13) has recently described in the heart of the salemperter, a South American lizard, a "sinu-auricular bundle" of specialised muscle, connecting the sinus with the specialised tissue lying in the floor of the auricle. Although from my earlier studies of the lizard and tortoise hearts it was certain that such a bundle did not exist in the hearts of the reptiles examined by me, this publication of Mackenzie's induced me again to go over my preparations, to which had been added in the meantime sections of the heart of the fence lizard (*Sceloporus undulatus*) and of the spotted tortoise (*Chelopus guttatus*). A part of this later material was fixed and stained by the same methods as were earlier used ('13 b) -fixation in strong Flem-

ming or in concentrated corrosive sublimate, and staining with iron hematoxylin and picric acid fuchsin. The remainder consisted of sections of hearts treated with methylen blue according to various methods, by Cajal's double impregnation method, as given by Hofmann ('02), and by the silver reduction method, as given by Meikeljohn ('13).

From this further study of these lizard and tortoise hearts no doubt has been thrown on the truth of the statement that the dorsal ligament is here a sino-ventricular bundle. But from Mackenzie's descriptions and from his figures it can also not be doubted that in the heart of the salemporter conditions are different. As he has himself pointed out, the conditions in this respect shown by the hearts of different reptiles are not the same and various stages can be recognized. We shall see that the conditions found in the hearts of the reptiles listed above represent still another stage in addition to those which he has described.

According to Mackenzie (p. 129) the "sinu-auricular ring" of the fish heart is represented in the heart of the salemporter "by a bundle or leash of fibres which lies in the groove between the left venous valve and the spatium intersepto-valvulare." The sinu-auricular bundle "courses round the posterior and under aspect of the sinus venosus just where the left duct of Cuvier enters the sinus and runs . . . a short distance as a free bundle to become continuous with the specialised tissue lying in the floor of the auricle, this tissue becoming in turn continuous with the auricular canal." In the heart of the crocodile (p. 130) the "sinu-auricular muscle" is "present at the base of the left venous valve at the junction of the sinus with the spatium. There is no direct continuity between the sinu-auricular bundle and the auriculo-ventricular bundle in the crocodile. The interruption takes place in the region of the sinus septum where the left duct of Cuvier enters the sinus." Later (p. 135) he goes on to say:

The sinu-auricular nodal tissue appears to become lost in this septum. It would appear that there are reptiles which in respect of this point exhibit an intermediate stage between the lizard (salemporter) and the crocodile. An example of this is the iguana, in which the sinu-auricular bundle is interrupted by a cord of fibrous tissue with iso-

lated muscle fibres and large nerve trunks. This cord occupies a corresponding position to the continuous muscle structure in *salearpenter* and in front appears again as a short isolated muscle bundle which in turn becomes continuous with the muscle of the auricular canal.

The conditions found in the lizard and tortoise hearts listed above represent another intermediate stage between that found in the iguana and that found in the crocodile, as described by Mackenzie. In these hearts there is no "isolated muscle bundle" which becomes continuous with the auricular canal. At the left venous valve, near its upper portion, there is, in the fibrous tissue a large group of nerve cells, which represents the endings of a branch of the right vagus nerve. The nerve fibers connected with these nerve cells can be followed for quite a distance along the right vein, as far as it is present in the sections, being connected with other large ganglia here and there along the vein. From this portion of the left valve there runs a band of connective tissue, a fold of the pericardium, which bending under the left vein becomes free from the dorsal surface. From this point it is continued downward as a free band, superficially over the dorsal surface of the right auricle to the ventricle, over the anterior dorsal surface of which it spreads, being wider at its point of attachment than elsewhere. Sometimes the bundle, before it reaches the ventricle, divides into two, or even three, parts, and often under these circumstances a fine branch can be seen bending still further to the right and running in the auricular-ventricular groove to the ventral side,

In the bundle there are numerous blood vessels and large nerve trunks with several groups of nerve cells. Sometimes these nerve cells are single and scattered, but there are also many large ganglia. By studying Mackenzie's figures of sagittal sections (plate 2, figs. 1-3) one sees on the dorsal surface of the hearts a mass of tissue which extends from the sinus region to the anterior dorsal portion of the ventricle. This tissue Mackenzie has not labelled, but it has a position very similar to the continuous sino-ventricular bundle in the hearts of the lizards and tortoises which I have studied. From figure 3 of this same plate of Mackenzie's, however, it is seen that the "sinu-auricu-

lar bundle" does go over directly into the auricular funnel musculature, the latter being, according to his representation, on the dorsal side a continuation of the "sinu-auricular bundle." A glance at the figures which are presented with this article will show that this is not the case in the animals with which we are dealing. The figures are untouched photographs of sagittal sections of the heart of the tortoise *Chelopus guttatus*. The hearts of the other tortoises and of the lizards show the same conditions. Drawings of the lizard heart have already been given (Laurens '13 b) and for that reason the tortoise is selected for the illustrations here.

After reaching the ventricle, the sino-ventricular bundle spreads out over its anterior dorsal surface. A portion of it runs to the back of the ventricle, while another portion goes down into the space between the funnel musculature and the inner wall of the ventricle. This space is filled with connective tissue containing blood vessels, nerves and ganglia (Laurens '13 b, fig. 4), and the portion of the sino-ventricular bundle which goes down into this space becomes continuous with this connective tissue, which is also, of course, a portion of the pericardium. The nerves in the sino-ventricular bundle, two of which are shown in figure 6, are also distributed, some of them to the anterior dorsal surface of the ventricle, and some to the connective tissue filled space between the funnel musculature and the inner wall of the ventricle. The latter innervate the auriculo-ventricular funnel and also supply the inner wall of the ventricle. Quite often the nerves in the sino-ventricular bundle are insignificant, and even entirely lacking, a fact which was also noted by Gaskell.

There is no muscle tissue, either continuous or isolated, in the sino-ventricular bundle. At its beginning (sinus end) and ending (ventricular end) a few isolated striated muscle fibers can sometimes be seen in the connective tissue (as in the sections from which figures 3 and 4 are taken). But it is clear that these muscle fibers have been pulled into this position by the knife tearing them away from the walls of the sinus and of the ventricle. In its free course there is only fibrous tissue in the bundle, in which nerve fibers, ganglion cells and blood vessels are found.

As the sino-ventricular bundle is followed in transverse and sagittal sections it is seen to be nothing more than a portion of the pericardium which is folded off as a free band to run between the sinus and the ventricle. In some places it is even connected with the pericardium proper over the right auricle by fine strands (fig. 5). By studying the figures, which represent sections in order from left to right, the manner in which the sino-ventricular bundle is folded off from the continuous pericardium can be made out. Figures 1 and 2 are sections to the left of the median line, and the dorsal ligament does not show at all. In figure 2, however, one of the large nerve trunks is seen running from the sinus, under the pericardium on the dorsal side of the auricle, to the ventricle across the auriculo-ventricular groove (Laurens '13 b and Dogiel and Archangelsky '06) to end in the auriculo-ventricular funnel musculature, after it has gone through the connective tissue in the space between the funnel and the ventricle. In figure 3 we see the beginning of the free portion of the sino-ventricular bundle in an out-folding of the pericardium on the anterior dorsal surface of the ventricle, and opposite to it a corresponding outfolding on the sinus wall. In figure 4 these folds have advanced further and in figure 5 they have met to form the continuous band of connective tissue, which can here be followed up along the sinus until bending over to the right it disappears from the section. Figure 6 gives another view of the sino-ventricular bundle further to the right. In this section a large nerve trunk is seen in the ligament coming from the sinus and going down into the connective tissue filling the space between the funnel and the ventricle. It also shows to the extreme right a portion of a smaller nerve going over to the outer wall of the ventricle. Figures 7 and 8 serve to illustrate the appearance of the ligament further over to the right hand side of the heart. From a study of these figures it will be clear, I believe, that the dorsal ligament is simply a fold of the pericardium, which, retaining its connection with the sinus and with the ventricle, runs free over the dorsal surface of the right auricle from the sinus to the ventricle, and is therefore strictly a sino-ventricular bundle.

THE SINO-AURICULAR CONNECTION

The connection between the sinus and the right auricle is a direct muscular one in the reptile hearts described in this paper. Gaskell pointed out the fact that this was the case in the tortoise with which he was working, though the details concerning the manner in which the connection was actually brought about do not hold here. Külbs and Lange ('10) also describe a direct muscular connection between the sinus and the right auricle in the lizard. But in the heart of the salemporter (Mackenzie) the ring of specialized muscle with numerous nerve cells and fibers at the 'sinu-auricular junction' in the fish is represented by a "bundle or leash of fibers which lies in the groove between the left venous valve and the spatium intersepto-valvulare." In the reptile hearts that I have examined there is a complete muscular ring. Nerve cells, in larger and smaller ganglia, and nerve fibers are all around this ring in the connective tissue. The musculature of the sinus goes over into that of the right auricle in much the same way that the musculature of the auriculo-ventricular funnel goes over into that of the ventricle. At the junction of the sinus with the auricle there are the two valves which completely close the oval shaped opening which runs obliquely from the upper right hand side to the lower left, as Mackenzie shows in his figure on plate 3. At the right, or lower, valve, the musculature of the sinus goes over into that of the auricle at the free edge, the two kinds of musculature being here continuous. At the left, or upper, valve the conditions are somewhat different. In its upper portion the valve is a continuation of the wall of that portion of the sinus, and the musculature of the sinus joins directly with that of the auricle along the valve (fig. 7). But the extreme lower portion of the left valve, which, in sagittal sections taken from the left to the right comes first into view, is formed from a portion of the auricular septum, being really a continuation of it (fig. 3), and the wall of the sinus here goes directly over into this portion of the septum, the left valve being here separated from the wall of the auricle by a layer of fibrous tissue, a condition particularly clearly shown in transverse sections.

THE AURICULO-VENTRICULAR CONNECTION

Attention may be here again called to this connection in the hearts of lizards and tortoises, since, from Mackenzie's description, there appear to be slight differences between the conditions found in the salempenter and those found in other lizards and in tortoises. In the salempenter, the auricular canal, according to Mackenzie, is specialized, an assumption also made by Külbs and Lange ('10) for the lizard (*L. viridis* and *L. muralis*) and by Külbs ('12 and '13) for the lizard and tortoise. It has already been pointed out (Laurens '13 b) that the musculature of the auriculo-ventricular funnel is not very different from the musculature of other portions of the auricles. The fibers and nuclei are similar to those of the auricles, though there is more sarcoplasm and fewer fibrillae. However, the funnel musculature is richly supplied with nerves and contains numerous capillaries, and in between its fibers, which are arranged circularly, there is a considerable amount of connective tissue. The striation of the fibers is distinct but fine, and is quite similar to that of the auricles. The striation of the ventricular fibers is coarser and the nuclei are much elongated and narrower than are those of the auricles and of the funnel.

The function of the auriculo-ventricular funnel in co-ordinating the contractions of the auricles and of the ventricle was very carefully worked out in the lizards, *L. viridis* and *L. agilis*, and in the tortoise, *Clemmys lutaria*. From this work (Laurens '13 a) it is evident that there is here a physiological differentiation in that certain portions of the funnel are more efficient than others in allowing the passage of the contraction wave from the auricles to the ventricle, and furthermore, in preserving the co-ordination of ventricular with auricular beat, when other portions of the connection between these parts of the heart are cut away. The portions showing this greater efficiency are the right and left sides of the funnel. Later (Laurens '13 b) it was shown that this physiological specialization had an anatomical basis in that, at these two portions, there was a more intimate connection between the funnel musculature and the musculature of the ventricle.

In the salemporter (Mackenzie, p. 129) the auricular canal is described as "a tube invaginated into the ventricle, becoming at its lower end continuous with the ventricular musculature in the region of the papillary muscles to which the auriculo-ventricular valves are attached. This invagination does not of course occur at that part of the orifice where the auricular canal is continued on to the bulbus musculature." In all the lizard and tortoise hearts studied by me the invagination does take place around the whole circumference of the orifice, the funnel being only broken through at its entrance into the ventricle, by the bulbus with the musculature of which the funnel musculature becomes continuous. The direct continuity between the musculature of the funnel and that of the ventricle does not, of course, occur at this place.

Mangold ('14) points out that on the dorsal side the funnel musculature in the salemporter, as described by Mackenzie, extends further into the cavity of the ventricle, before the fusion of the two kinds of musculature takes place, than it does in the hearts of the reptiles which were described by me, where it very soon becomes broken through by its fusion with the ventricle. This difference, however, is I think, very slight. By comparing Mackenzie's figures with mine it will readily be seen that the length of the ventricle of the salemporter is relatively, when compared with its dorso-ventral thickness, less than that of the lizards and of the tortoises here described. Moreover, that the attachment of the auriculo-ventricular valves as represented in the salemporter is much nearer the apex, and that the auriculo-ventricular funnel extends further into the cavity of the ventricle, than in the other lizards and tortoises. From a glance at figure 4 (Laurens '13 b) it will be apparent that on the dorsal side the funnel musculature is continued, although quite thin, almost to the attachment of the auriculo-ventricular valves to the papillary muscles, and the figures presented with the present article show this quite plainly. On the right and left sides, however, the funnel musculature is continued further into the ventricle, before the fusion between the two kinds of musculature finally takes place, the connection at these parts being

therefore more intimate than at other portions. Mackenzie does not mention whether the final continuity between the auriculo-ventricular funnel and the ventricle takes place at all portions at the same level.

There is one other matter concerning the auriculo-ventricular connection, and that is its innervation. Nerve fibers can be seen extending downward from the sinus in the pericardium and can be followed across the auriculo-ventricular groove. For the past two years I have been studying the innervation of the reptile heart, and particularly of the auriculo-ventricular funnel muscle, by means of the special methods mentioned earlier. Although perfect results have not yet been obtained, it has been seen that the auriculo-ventricular funnel is richly supplied with nerves, in the form of a net-work of fine fibers, which come to it from branches of nerves descending along the back of the auricles in the way described earlier by me ('13 b), and by Dogiel and Archangelsky ('06). Nerve fibers and cells are also found on the inside of the auricles, running along the inner edge of the walls and along the septum, and which come into the heart along or near the entrance of the pulmonary vein and of the left duct of Cuvier. These nerves also give off branches which run down between the auriculo-ventricular valves and the inner side of the funnel to finally become distributed to the latter. In the funnel musculature itself, nerve cells are scarce, only a few scattered ones being found here and there. But in the connective tissue of the groove, and of the space between the funnel and the inner wall of the ventricle, ganglia are numerous, particularly on the dorsal side, though in sections of some hearts the number of ganglia found on the ventral side, especially near the bulbus, and on the right and left sides of the funnel is also quite large.

The nerves which run over the dorsal surface of the auricles and of the auriculo-ventricular groove to be continued into the connective tissue between the funnel and the ventricle can also be easily seen in sagittal sections of material fixed in Flemming and in corrosive sublimate. In figure 2 one of these nerves is shown running down to finally become distributed to the auriculo-ventricular funnel musculature (see also fig. 6).

THE PROBABLE FUNCTION AND FATE OF THE SINO-VENTRICULAR BUNDLE

The sino-ventricular bundle, contrary to the view of Imchanitzky ('09), has nothing to do with the coördination of ventricular and auricular contraction (Gaskell '84 and Laurens '13 a). Furthermore it has no function in bringing about a possible sino-ventricular rhythm. In the salemphenter, however, according to Mackenzie, 'the sinu-auricular bundle' is made up of specialized muscle. In support of which statement, we have no physiological evidence.

The contraction wave begins in the walls of the sinus and spreads to the auricles along the sino-auricular junction, and from there along the auriculo-ventricular funnel to the ventricle. Can the 'sinu-auricular bundle' in the salemphenter be a pathway for impulses passing direct from the sinus to the auriculo-ventricular connection and to the ventricle, and if so, what is the nature of these impulses? That they have anything to do with coördination can hardly be claimed owing to the physiological evidence against such an assumption. Gaskell (p. 83) showed that when the peripheral end of the 'coronary nerve' was stimulated that the rate of beat of the heart was not changed, although when the central end was stimulated a decided slowing of auricular rate took place. By the stimulation of either end of the nerve the force of the auricular contractions was diminished (p. 92). Further, Gaskell showed (p. 85) that, when the connection between the sinus and the auricles is severed so that the 'coronary nerve' remains intact and as the only connection between the auricle and ventricle and the body of the animal and an independent auriculo-ventricular rhythm is set up, stimulation of the right vagus brings about a decrease in the force of the auricular contractions alone. In one experiment, out of several, Gaskell obtained an inhibition of this independent auriculo-ventricular rhythm, when the right vagus nerve was stimulated. His explanation of this was that "when the coronary nerve happens to contain fibers which supply the particular muscles which originate the independent rhythm,

then stimulation of the vagus can inhibit that rhythm." Moreover, as Gaskell also pointed out, the 'coronary nerve' is only one of several nerve trunks, branches of the vagus nerves, which pass from the sinus to the ventricle and to the auriculo-ventricular funnel musculature, and it is therefore but natural that the auriculo-ventricular rhythm should be controlled by it when it happens to innervate the muscles which originate this rhythm, just as the other branches of the vagus nerves do.

The 'coronary nerve,' when the vagus is stimulated, is also no more able to conduct an impulse to the ventricle by which its force of contraction can be diminished, than are any of the other nerve trunks passing from the sinus to the ventricle (Gaskell, p. 91). It has also another function in common with the other branches of the vagus nerves in that it carries impulses, upon stimulation of the vagus which improve the conduction power of a strip between the auricle and the ventricle during a condition of partial block, though it may also have a function occasionally in increasing such a block by diminishing the conducting power of the connecting strip (Gaskell, p. 96).

The 'coronary nerve' is not always present, and is often very insignificant. From what has just been said it apparently has no particular function that the other nerve trunks do not have, and we are forced to the conclusion that its function is relatively unimportant, or perhaps better, no more important than that of the other branches of the vagus nerves.

From physiological evidence then, the effect of the 'coronary nerve,' like the other branches of the vagus nerves, are on the auricles, and it may be assumed to have an additional regulatory effect when, for any reason, the rhythm producing power of the auriculo-ventricular funnel is brought into prominence above that of the sinus. As we have seen, some of the nerve trunks which run in the sino-ventricular bundle go into the connective tissue between the funnel and the ventricle, which is a condition that was not realised at the time of my earlier publication. It is highly probable that the nerves of the 'sinu-auricular bundle' of the salemporter have the same function as the 'coro-

nary nerve' in the heart of the tortoise described by Gaskell. But what the function of the 'specialized muscle' of this bundle is, must remain for the present an unanswered question.

As to the probable fate of the sino-ventricular bundle, Mackenzie has given the steps leading up to the condition found in the reptile hearts described in the present article. In the salamenter there is a "sinu-auricular bundle of specialised muscle;" in the iguana the bundle is interrupted by a cord of fibrous tissue, a short isolated muscle bundle, which is continuous with the muscle of the auricular canal, being all that is left. In the hearts of the lizards and tortoises here described, the sino-auricular junction is a ring of muscle completely surrounding the opening of the sinus into the right auricle, and running from this region there is a band of connective tissue which goes to the anterior dorsal surface of the ventricle. In this band of connective tissue there are large nerve trunks and blood vessels some of which go to the ventricle, others into the connective tissue between the funnel and inner wall of the ventricle to become distributed to the musculature of both. In the crocodile, all that there is of the 'sinu-auricular muscle' according to Mackenzie, is at the base of the left venous valve at the junction of the sinus with the spatium.

Mackenzie speculates on the probable fate of the fibrous cord and of the distal isolated muscle bundle found in the iguana. He considers it "not improbable that it becomes incorporated in the auricular floor, and that it is represented in the higher reptilian and mammalian hearts by a bundle of fibrous tissue which runs in the basal wall of the auricle at the line of attachment of the septum between the region of the coronary sinus and the septum fibrosum." As we have seen, however, in the hearts of other lizards and tortoises; the 'distal muscular bundle' has disappeared, while the 'fibrous cord' is still present as a sino-ventricular bundle.

In this connection attention may be called to the recent publication of Stanley Kent in which it has been shown that in man "the auriculo-ventricular bundle is not the only part by which the functional connection between the auricle and ven-

tricle may be established," (Kent '14 a). In a series of papers Kent describes and figures a specialized tissue on the right lateral aspect of the heart, as a neuro-muscular connection between the auricle and the ventricle, with large nerve trunks in the fat and connective tissue of the groove, and also in the muscular tissue. He also shows ('14 c) that, when all other connections between the auricles and ventricles are severed, contractions of the auricles are still carried over to the ventricles. This connection, according to Kent ('14 a), may constitute a "local reflex arc which may perhaps exhibit only an occasional activity," and "which may be capable of controlling . . . coördination when the bundle is no longer perfect."

Whether the 'sinu-auricular bundle' of the salemponenter, and the sino-ventricular bundle of *Lacerta agilis*, *L. viridis*, *Sceloporus undulatus*, and of *Clemmys lutaria*, *Chelopus in sculptus* and *C. guttatus* have anything in common with this bundle described by Kent is of course a question for the future.

SUMMARY

1. The dorsal ligament in the hearts of *Lacerta viridis*, *L. agilis*, *Sceloporus undulatus*, and of *Clemmys lutaria*, *Chelopus in sculptus* and *C. guttatus* is a sino-ventricular bundle of connective tissue, a fold of the pericardium, which, extending from the sino-auricular junction near the upper portion of the left venous valve, runs under the left vein and is continued as a free bundle to the ventricle on the anterior dorsal surface of which it ends; there becoming continuous again with the pericardium. In this bundle there are large nerve trunks and blood vessels. Some of the nerves go to the dorsal surface of the ventricle, others to the auriculo-ventricular funnel, after they have gone down into the connective tissue filling the space between the musculature of the auriculo-ventricular funnel and the inner wall of the ventricle, and still others to the inner wall of the ventricle.

2. The sino-auricular junction is in the form of an oval-shaped muscular ring. At the right valve the musculature of

the sinus and that of the auricle is continuous. At the left valve in its upper portion the musculature of the two chambers is also continuous, but in its lower portion the musculature of the sinus goes over into that of the auricular septum which forms this portion of the valve, there being here a layer of connective tissue between the left valve and the wall of the auricle.

3. The auriculo-ventricular junction has in cross section the form of a ring; in sagittal section it is in the form of a funnel which extends down into the orifice of the ventricle, with the musculature of which it gradually becomes continuous. On the right ventral side the ring is interrupted by the bulbus with the walls of which it also becomes continuous. The fusion between the funnel and ventricle musculature takes place at the lower level of the auriculo-ventricular valves near their attachment to the papillary muscles. The dorsal portion of the funnel is the first to become continuous with the ventricle, while the right and left sides are the last.

4. The auriculo-ventricular funnel is richly innervated by branches of the right and left vagus nerves which, coursing along the dorsal surface of the auricles, under the pericardium, cross the auriculo-ventricular groove, in the connective tissue of which there are many ganglion cells, go down into the connective tissue filled space between the funnel and the inner wall of the ventricle, and finally end in the funnel musculature and in the musculature of the ventricle. There are also a few nerves on the ventral side, and a few which course along the inner walls of the auricles and the auricular septum to end in the musculature of the funnel.

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EXPLANATION OF PLATES

All the figures are untouched photographs of sagittal sections of the heart of the tortoise *Chelopus guttatus*. They are arranged in order from the left to the right hand side of the heart. In all of them the auriculo-ventricular funnel is shown invaginated into the cavity of the ventricle to become continuous with its musculature near the attachment of the auriculo-ventricular valves.

PLATE 1

EXPLANATION OF FIGURES

1 A section through the left auricle passing through the right side of the opening of the pulmonary vein into the left auricle; a portion of the auricular septum is seen.

2 A section to the right of the opening of the pulmonary vein. On the dorsal side a nerve trunk can be seen running in the pericardium to end in the musculature of the auricular-ventricular funnel.

3 A section to the left of the opening of the sinus into the right auricle. On the dorsal side the beginnings of the folding of the pericardium, which from the sino-ventricular bundle, are shown.

4 A section just to the left of the opening of the sinus; the folds of the pericardium have advanced further; the two venous valves are seen.

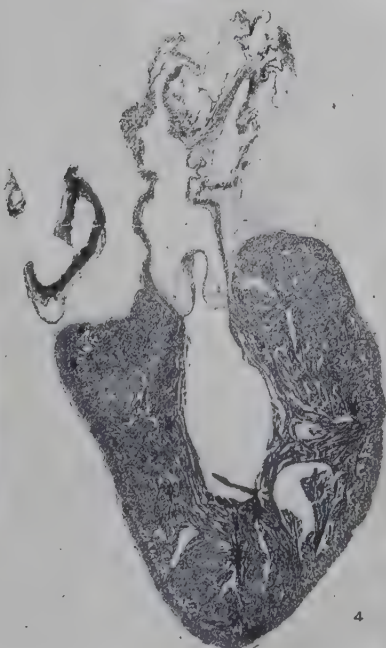
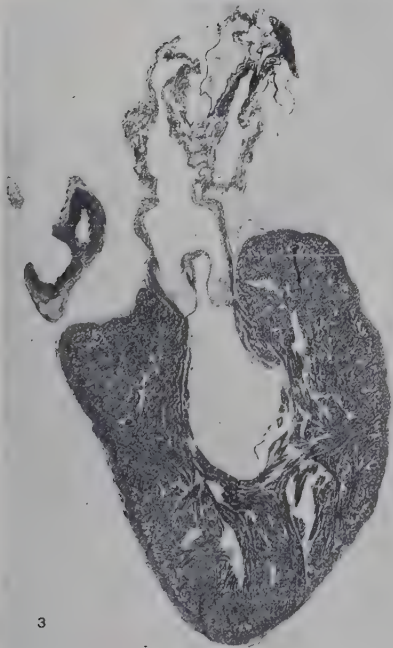
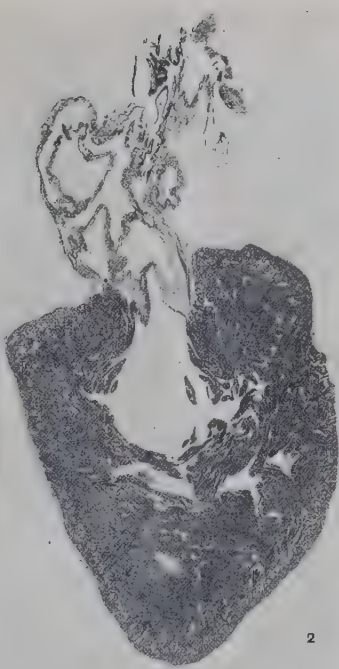


PLATE 2

EXPLANATION OF FIGURES

5 A section through the opening of the sinus into the right auricle. The sino-ventricular bundle is here seen extending as a free band over the auricle from the sinus to the anterior dorsal surface of the ventricle. Just above and to the right of where it joins the sinus there is a group of nerve cells and fibers, and further anterior and to the right, another group.

6 A section further to the right than figure 5. In the sino-ventricular bundle two nerve trunks can be seen; a stronger one running down into the connective tissue of the space between the funnel and the inner wall of the ventricle, and a smaller one, to the right, going to the outer surface of the ventricle.

7 and 8 Sections far over to the right of the auriculo-ventricular connection to show the fold of the pericardium which forms the sino-ventricular bundle becoming continuous with the pericardium over the sinus, the auricle, and the ventricle.



THE ADULT ANATOMY OF THE LYMPHATIC SYSTEM IN THE COMMON RAT (*EPIMYS NORVEGICUS*)¹

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FOUR FIGURES

In the fall of 1914 the writer undertook the study of the origin and development of the lymphatic system in the common rat. Before the work had proceeded very far it was evident that a knowledge of the adult anatomy would be of no small amount of help in guiding and interpreting the work on the origin and development of this system. So the plan was changed to a study of the adult anatomy of the lymphatic system. This paper gives the chief results of the work.

Fifty rats have been examined in the course of the study. The lymphatic system was injected from the soles of the feet, the tip of the tongue, the lips, the walls of the intestines, the spleen, the lumbar, thoracic, inguinal, axial, intestinal and cervical lymph nodes. The first nineteen specimens were injected with India ink, using the hypodermic syringe for pressure. In the next six specimens, a solution of soluble blue was used, with a glass cannula and pressure bulb apparatus. The remainder of the work was done with Berlin blue gelatin mass, a small crystal each of thymol and potassium iodide being added to preserve and lower the melting point of the mass. The supply of gelatin mass was kept in a warm water bath, and the cannula occasionally warmed. Injections could be made through a much smaller aperture and in a much more satisfactory way by this method.

¹ An abstract of a thesis presented to the Graduate Faculty of the State University of Iowa for the degree of Master of Science.

RESULTS

Injections made through the soles of the feet, showed a variable number of superficial lymph vessels joining to form larger lymph vessels which followed the main course of the radial and ulnar veins, or dorsal and plantar branches of the saphenous vein, to the elbow or knee lymph node (1, 2, 3, 4, fig. 1). From the single node, 3 and 4, the femoral lymph vessel continued to the lumbar node (5-6). A branch is given off from this vessel at the juncture of the superficial epigastric vein and the femoral vein, which leads to the inguinal nodes (7-8).

Just below the posterior end of the rectum is a small single node (30) which receives the lymph from the caudal region and appendages. From this node a vessel leads to the left lumbar node (6), or to the left femoral lymph vessel, joining it at about the juncture of the iliac and inferior vena cava.

The lumbar nodes (5-6), which are normally double, lie just caudad of the ilio-lumbar veins, on either side of the vena cava. There is a great range of variation, however, in the position of these nodes, due, in the main, to the variability of the ilio-lumbar veins. If the veins are well caudad, the lymph nodes may be crowded back opposite to each other; or, if the veins are well

FIGURE 1

- 1-2, right and left elbow nodes
- 3-4, right and left knee nodes
- 5-6, lumbar nodes
- 7-8, inguinal nodes
- 9-10, renal nodes
- 11, cisterna chyli
- 12, cisterna group of lymph nodes
- 13, intestinal node
- 14, thoracic duct
- 15 to 20, axial nodes
- 23-24, jugulo-subclavian taps
- 25, thoracic group
- 26, posterior cervical nodes
- 27, anterior cervical nodes
- 28, submaxillary nodes
- 29, tongue and lip plexus
- 30, caudal lymph node

FIGURES 2, 3

- a*, renal lymph vessel
- ci*, cisterna chyli; *12*, cisterna group
- 13*, intestinal node; *5-6*, lumbar nodes
- 9-10*, renal nodes
- p.c.v.*, posterior vena cava
- i-l.*, ilio-lumbar vein

FIGURE 4

- 1, spleen; 2, fundus lymph vessel
- 3, pyloric lymph vessel
- 4, intestinal lymph vessel
- 5, connection with portal vein
- 6, intestinal branch to cisterna chyli
- 7, appendix; 8, mesenteric nodes
- 9, intestinal vessel
- 10, mesenteric branches of lymph vessel
- 13, intestinal node

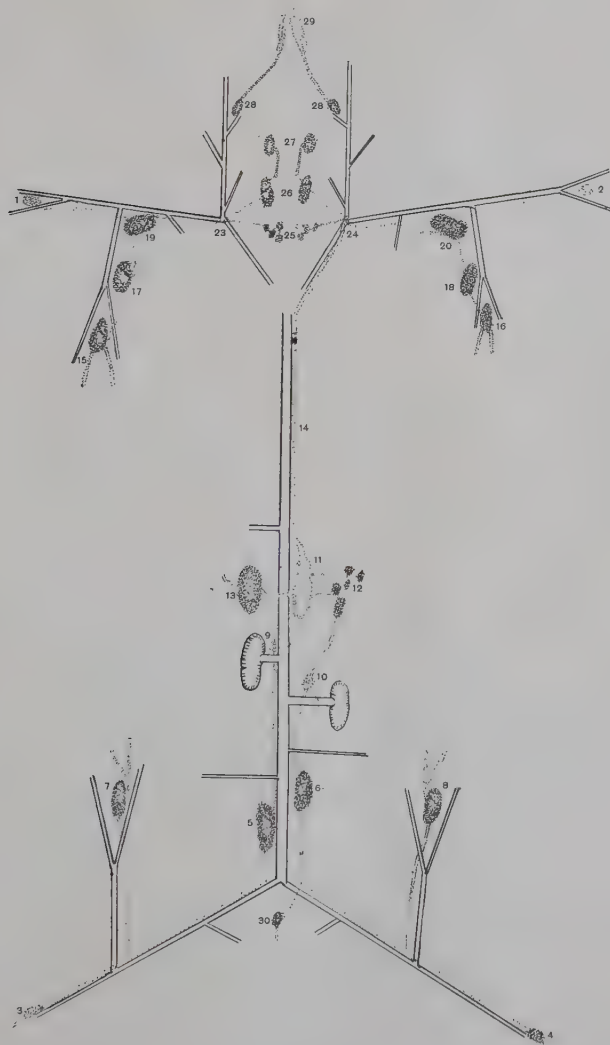
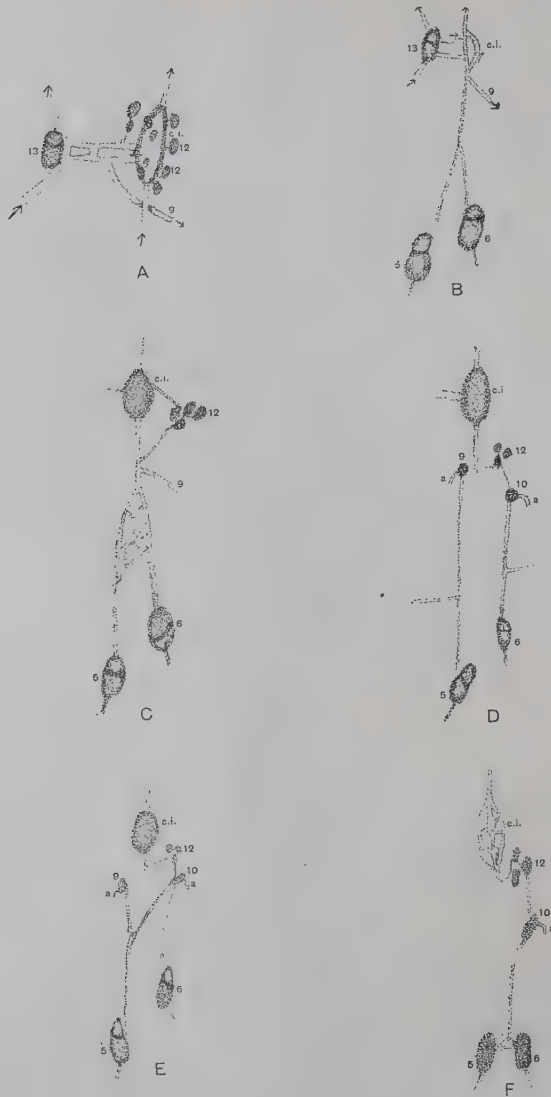
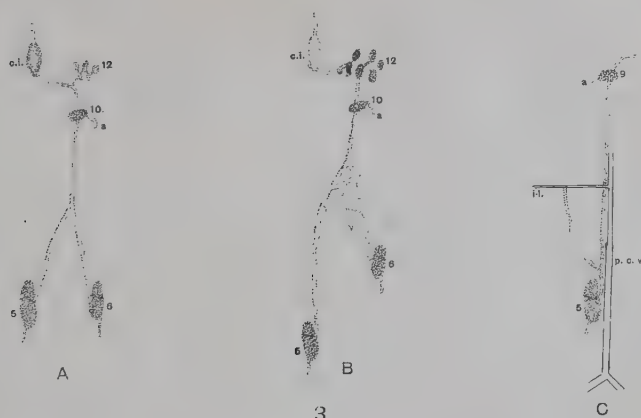


Fig. 1 Venous system shown diagrammatically in solid lines, the lymphatic system stippled. The lateral veins and lymph vessels are continuous, although not shown so in the figure. Lymph node 13, the intestinal node, so marked in all drawings.





Figs. 2, 3 Exceptional and type variations of the lumbar region

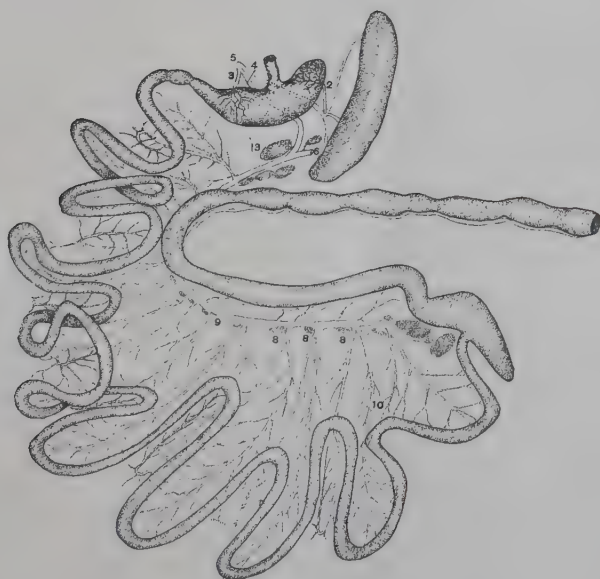


Fig. 4 The intestinal tract and lymphatics

forward, the left lumbar node may be a considerable distance in advance of the right node. In a few instances the double nodes were divided into two single nodes, which lie some distance apart, and in two specimens an additional double node was found at the bifurcation of the ilio-lumbar vein on the right side.

The lymph vessel leading from the right lymph node (5) is so variable that a definite description cannot be given. It usually joins the left lymph vessel in some way between the lumbar node and the cisterna chyli. Figure 2, D, E, F, shows some variations of a general type. Figure 3, A and B, shows two additional variations, which are more common, in the general plan, than any of the others. In only four specimens did the right lumbar lymph vessel lead to the right renal node (9) directly. In these cases a small lymph vessel tapped the renal vein from the renal node, and another vessel connected with the cisterna chyli (11), or the cisterna chyli group (12). In two specimens the right lumbar lymph vessel gave off a branch, which connected with the ilio-lumbar vein (fig. 3, C). This branch did not follow the veins to its tap, but went directly from the node across the ilio-psoas and psoas muscles to its juncture with the ilio-lumbar vein about 1 cm. from the vena cava.

On the left side the number of lumbar lymph vessels leading from the lumbar node (6) may vary from one to four, or form a network, depending somewhat on the mode of attachment with the right lymph vessel. However, all the vessels lead along the left side of the vena cava, in any case. If there is only one vessel, it will open into a single node (10), just anterior to the left renal vein, from which a branch is given to the renal vein and one to the group of single nodes (12) lying to the left of the cisterna chyli. If there be more than one lymph vessel leaving the lumbar node, some one of them will enter the renal node, the rest may join the cisterna group, the cisterna directly, the renal vein directly, or any combination thereof. The latter conditions are fewer than the single vessel method.

The number of nodes in the cisterna group (12) vary from one large one to six small ones. From this group one or more

vessels enter the cisterna chyli. Two special exceptions to this method are shown in A and B of figure 2. Drawing A shows both of the lumbar lymph vessels entering the cisterna chyli directly. The nodes of the cisterna group being closely collected about the periphery of the cisterna chyli, did not fill with the injection mass. The connection with the left renal vein was made from the intestinal lymph vessel (13) through a small branch vessel (*a*). The specimen from which drawing B was made, had no cisterna chyli, only a short vessel shunted off from the main thoracic duct marked its normal position (*ci*). The renal branch (*a*) came from the main lumbar lymph vessel. In both of these specimens the connection between the intestinal lymphatics and the portal vein was very prominent.

In addition to the connections with the cisterna chyli already mentioned, there is another lymph vessel received from the intestinal region past the intestinal node (13). The intestinal lymph does not pass through the intestinal node, but that the node is connected with the lymphatic system is shown by the fact that injections made from it fill the main lymph vessels. Also, the mass from the lumbar injections frequently pass into this node but not beyond it. From the cisterna chyli the thoracic duct (14) leads dorso-laterally along the superior vena cava and left innominate vein to its juncture with the venous system in the jugulo-subclavian district. Not a single specimen in the fifty rats showed the thoracic duct branching or entering the right jugulo-subclavian district, as pointed out by McClure and Silvester,² Silvester,³ and Davis,⁴ in other forms.

A double lymph node is found in the groin, the inguinal nodes (7-8), located in the bifurcations of the superficial epigastric vein, and so closely attached to the lower dermis that in removing the skin the node remains imbedded in the subcutaneous

² McClure and Silvester. A comparative study of the lymphatico-venous communications in adult mammals. *Anat. Rec.*, vol. 3, 1909.

³ F. Silvester. On the presence of permanent communications between the lymphatic and venous system at the level of the renal vein in adult South American monkeys. *Am. Jour. Anat.*, vol. 12, 1912.

⁴ Henry K. Davis. A statical study of the thoracic duct in man. *Am. Jour. Anat.*, vol. 17, 1915.

tissue. While this node is connected with the femoral lymph vessel, in only two injections did the mass run backward into it. The vessel leaving anteriorly from the inguinal node soon divides into two branches. These branches lead through the lower layers of the dermis to the axial region where they again join to enter a double node, which is usually found in the bifurcation of the lateral thoracic vein (15-16). From here a lymph vessel leads to another double node (17-18) located in the same general region only a little anteriorly; then to the third double node of this group (19-20) found by the intersection of the lateral thoracic and the axillary vein. This node also receives the lymph vessels from the elbow nodes (1-2). On the left side a lymph vessel leads from node 20 to the communication with the venous system, either through the thoracic duct tap (24), or through a separate tap in the immediate district. On the right side the communication is in the same relative position (23).

In the anterior part of the thorax, between the two innominate veins, are two groups of single nodes, the thoracic groups (25). Each group varies in the number of nodes it contains, from four to eight. When one node in the group is injected all the other nodes of that group fill up with the mass and show a vessel leading to the venous connection in the jugulo-subclavian district. The right group connects with the right district and the left with the left district. Injections from other parts of the lymphatic system do not show these nodes.

The tongue and lips have many small vessels which collect in larger main vessels on each side of the head. These vessels lead to the submaxillary lymph nodes (28), which lie at the branching of the external jugular vein into the anterior facial and transverse vein. From these nodes, vessels lead almost directly inward, dorsally, to single nodes lying on each side of the trachea. Further down the trachea, on each side, are two nodes lying in close proximity, one a very small single node and the other a large double node. A lymph vessel leads from the larger node to the jugulo-subclavian tap on its respective side.

From injections in the intestinal walls a great number of small vessels are shown collecting into larger and larger vessels until they finally join the great intestinal lymph vessel, which follows the large intestine from the appendix to the intestinal lymph node in the anterior part of the abdominal cavity. In the region of the appendix there are several lymph nodes, all of which are single. In some cases they are so united as to form one large compound node, 2 or 3 cm. long. There is usually a single node at the juncture of each mesenteric lymph vessel (8, fig. 4) with the main intestinal lymph vessel (9, fig. 4). Just a short way from the cisterna chyli is the large, single, intestinal node (13, figs. 1, 2, 4), which marks the branching of the large intestinal lymph vessel. From here one branch goes to the cisterna chyli (6) and the other to the portal vein (5). The injections made from the nodes in the region of the appendix did not always show the portal branch of the vessel, but there were a sufficient number of cases to demonstrate that such a connection is fairly common. There were a varying number of single nodes in the region of the large intestinal node which did not fill up with the mass from the intestinal injection. However, injections made from them showed that they were connected with the intestinal lymph vessels.

Injections made from the spleen (1, fig. 4) show four lymph vessels leaving at various places along the hilus, accompanied by the splenic veins. Near the cardiac end of the stomach the four splenic lymph vessels unite with a small lymph vessel from the fundus (2) to form the main splenic lymph vessel which now proceeds dorsally toward the pyloric end of the stomach, from which it receives another small lymph vessel (3). Almost immediately thereafter, the branch from the intestinal lymphatics, when present (4), and the splenic vessel unite to join the portal vein (5).

REMARKS

On the nodes. There seem to be two types of nodes; (a) a single node in which the lymph enters at the periphery, passes through the body of the node to the hilus, where a lymph vessel is formed; (b) the double node, referred to above, in which two single nodes are bound together in the same capsule. Instances showing this double nature are seen when injections made distal⁵ to a double node fill the posterior half of the node and pass on well into the vessels beyond before the anterior half of the node begins to fill; injections made in the posterior half of these nodes will not fill the anterior half for some time after the vessel leading from the node is filled; injections made in the anterior half seldom enters the posterior half; in some instances where normally a double node is found, two single nodes were found only slightly separated; and finally, dissections can usually be made, to show the double nature of the nodes. The significance of the double node has not yet been determined, but it is possible that the two parts perform separate functions. The posterior part of the node responds to the injection mass, just as the single nodes of the head, knee, elbow and caudal region do, while the anterior part responds as the intestinal and thoracic nodes. It is possible, therefore, that the posterior part and certain single nodes may act as filters primarily, while the anterior part and certain other single nodes may act as elaborators only.⁶ A histological study is to follow.

On the vessels. No attempt was made in this study to determine the nature of the origin of the lymph vessels in the tissues. The injections do show, however, a definite tubed system beyond the origin, carrying lymph in only one direction and that toward the venous connection. The valves in the vessels are shown by the knotted appearance of those vessels filled with the mass. In general, the lymph vessels follow the blood vessels very closely, the lumbar region being the main exception. The walls are

⁵ The venous connection is considered the anterior end of the lymph vessel.

⁶ Sabin, in Morris's "Human anatomy," Part II, page 702, recognizes one type of lymph node with two functions.

very thin and easily ruptured by mechanical devices, but are capable of considerable extension in situ without injury. Where a lymph vessel passes through a node it always enters at the periphery and leaves from the hilus.

On the connections. In addition to the venous connections pointed out by McClure,² Silvester,³ and others, there appears to be two additional connections in the rat, the *portal vein connection* and the *ilio-lumbar connection*.

The portal vein connection receives the lymph from the spleen and stomach, and part of the intestinal lymph (fig. 4, 5).

The ilio-lumbar connection receives some lymph from the right lumbar lymph vessel (fig. 3, C).

The left jugulo-subclavian tap, studied by McClure and Silvester in several forms,² in the rat receives the lymph from the left side of the head and neck, the left thoracic group, the left fore limb, side and hind limb, the deep seated vessels of the right hind limb and lumbar region.

The right jugulo-subclavian tap² receives the lymph from the right side of the head and neck, the right thoracic group, the right fore limb and the superficial vessels of the right side and hind limb (fig. 1, 23).

The right renal vein communications, studied by Silvester,³ when present in the rat, receives part of the lymph from the deep seated vessels of the right hind limb (fig. 1, 9).

The left renal vein communication,³ receives part of the lymph from the hind quarters (fig. 1, 10).

The portal and ilio-lumbar vein connections have not been reported previously, to the knowledge of the writer.

On general arrangement. The lymphatic system of the trunk is sinistral in the rat. The irregular occurrence and the extremely variable connections of the right lumbar lymphatics, as contrasted with the comparatively stable left lumbar system, is evidence of this fact. In all of the appendages of the body the lymph vessels follow very closely the various branches of the venous system. When the lymphatic system is injected it is very easy to distinguish the arterial, venous and lymphatic systems, when they occur together.

In conclusion, I take this opportunity to thank Dr. Frank A. Stromsten for his many helpful suggestions in the course of this work, and for his human interest, which has made this work possible and a pleasure.

SOME ANATOMICAL DEDUCTIONS FROM A PATHOLOGICAL TEMPORO-MANDIBULAR ARTICULATION

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THREE FIGURES

In a previous paper, giving some observations on the jaw-joint,¹ I stated my belief that ordinarily the jaw is opened solely by the contraction of the two external pterygoid muscles; that the apparent barrier to the forward movement of the condyle, offered by the articular eminence, is almost entirely removed in life by the presence of the meniscus, or interarticular fibrocartilage, which, by its peculiar shape and movements largely obliterates the working depth of the glenoid fossa; and that thus during this action the path described by the moving condyle is almost a straight, rather than a curved, line, the direction of which is nearly parallel to the plane of pull of the lower heads of the two external pterygoid muscles.

A few months ago, through the courtesy of Prof. F. W. Putnam, Director of the Peabody Museum at Harvard College, I was given access to its splendid collection of several thousand skulls. In the course of my work on this material I came across a particular specimen, offering very direct, corroborative testimony on the points just noted, which I had demonstrated in my 'physiological' model two years ago, as well as to hint at one or two facts not mentioned before.

This skull was that of an adult negro from Algoa Bay; it was in good condition, and was normal except for the bony surfaces

¹ Observations on the temporo-mandibular articulation. *Anat. Rec.*, vol. 7, no. 10, pp. 355-367.

at the right temporo-mandibular articulation, and for a very slight modification in the left joint as well. The teeth were practically all present, were well worn and equally so on both sides, indicating that the two sides were used interchangeably in chewing. At the left jaw-joint there was a slight roughening of the posterior part of the articular eminence, as if its covering of hyaline cartilage had been eroded at that point, but elsewhere the bony surfaces appeared normal.

At the right joint, however, very extensive changes had taken place. The condyle was elongated anteriorly to double the antero-posterior diameter of that on the other side. This made the altered condylar process about as long as it was wide, being roughly the size of a twenty-five-cent piece, though not circular in outline. It was made up of two flat surfaces, one sloping down and laterally, the other down and mesially, from a central line, running in a sagittal plane the whole length of the process. It thus resembled an inverted trough. A transverse section gave an obtuse angle of about 120 degrees, and a longitudinal, or antero-posterior, section was a straight line. Its shape was then very different from that of the normal condyle as found on the other side (fig. 1).

The opposing surface on the temporal bone presented an appearance equally altered from the normal. Over the region of the original articular eminence and the anterior half of the glenoid fossa was a rough surface, the reverse of that noted on the condyle, being a wide trough with its two surfaces meeting also in a line running antero-posteriorly, the whole being somewhat longer in that direction than the corresponding condylar surface. Behind it was a part of the glenoid fossa, but evidently not utilized in any way as an articular surface (fig. 2).

Putting the jaw in its proper position, as determined by the perfect occlusion of the teeth, it was at once seen that the peculiar right condyle fitted exactly into the trough-like surface on the temporal bone, anterior to the remains of the old glenoid fossa, and that the left condyle was similarly advanced a short distance in front of its usual position. The motion on the left side, although restricted by the slightly advanced position of

the left condyle, was otherwise probably almost normal. On the right side the condyle moved in its socket, much as the carriage of a sliding microtome knife slides freely back and forth in its bearings, with no possible lateral deviation.

Obviously the right meniscus was either entirely lacking, or, if present, did not serve its ordinary function of adjusting the bones to each other, as shown by the shape of the articulating surfaces and the uniform thickness of the intervening space.

Nevertheless, as shown by the wear of the teeth, and the shape and relative size of the opposing surfaces of the enlarged condyle and its reverse, the former glenoid fossa and articular eminence, the condyle must have moved forward and backward during the various motions of the jaw very much as it did on the left side or as it does in a normal case. If there had been no forward and backward motion of the condyle, such as is necessary for proper trituration, the teeth could not have been so worn, and the articular surfaces would have had an entirely entirely different shape. With only a pure hinge movement the jaw-joint would undoubtedly have been more like that of the Carnivora, a cylinder rotating about its transverse axis, fixed in a cylindrical fossa above.

It is easy to see how the muscles of mastication in moving the jaw during the activity of the osteo-arthritis, which was evidently the cause of this abnormality, while the diseased surfaces were still plastic, formed joint surfaces exactly adapted to their proper sort of motion and to no other. The meniscus apparently had to be sacrificed, due to the disease, and certain advantages pertaining to this mechanism were lost, such as the lessened degree of friction in this moving hinge-joint and the presence of an elastic cushion to take up the shock of sudden and hard biting. But a very satisfactory substitute for a normal temporo-mandibular articulation was thus manufactured, a substitute that gave the same kinds of motion, though less in amplitude, as ordinarily found, in opening the jaw and in trituration, and yet one that accomplished this without the presence of a glenoid fossa, articular eminence, functioning meniscus, or cylindrical condyle.

Due to the peculiar conditions obtaining in this unusual specimen we have here crystallized evidence of what kind of motion was going on in that joint, a motion which must have been similar to that of the other, practically normal, side. The evidence is much better than that offered in the ordinary case where the presence of an active meniscus makes more difficult an appreciation of the actual path of the advancing condyle. Here the path itself is molded for us, not in moving cartilage, but in the enduring material of bone.

Study of this 'ossified testimony' gives us the following: The path of the advancing condyle was in a straight line, as noted in the working model described in my previous paper. There is no glenoid fossa or articular eminence in this case. So in the ordinary skull these irregularities are nearly smoothed out by the presence of the meniscus; and the condyle, there also, moves in a nearly straight line (fig. 3).

If the lines of the two advancing condyles be joined by a plane, that plane is seen to be almost identical with the plane formed similarly by the lines of pull of the lower heads of the two external pterygoids. The latter plane makes a slight angle with the former, about five degrees above its level, just enough to enable the opening muscles, the two external pterygoids, to apply the condyle not too firmly against the skull above, rather than to pull it away from its bearings, keeping the joint surfaces in close contact ready for the instant action of the closing muscles to take effect at any stage of the process of opening. It is interesting to note, however, that in closing the mouth the muscle which retracts the condyle, the posterior fibers of the temporal, does so at not less than an angle of twenty-five degrees above the plane of the condylar path. Thus it always keeps the bony surfaces in very firm contact.

The path of the condylar advance lies in the plane of the temporal muscle, very naturally, it seems, as the strongest of all the closing muscles, and also as the only retracting muscle. Very likely it is this muscle that had most to do with the molding of the plastic bony surfaces during the active stage of the disease. The condylar path, in being parallel to the sagittal

plane, is thus parallel to the resultant of the combined pull of the two external pterygoids, the main muscles utilized in opening the mouth.

Examining the condyle of the diseased joint by an imaginary coronal section its two surfaces are seen to slant as follows: the slope running laterally from the ridge at its summit makes an angle of about twenty degrees with the horizontal, while the

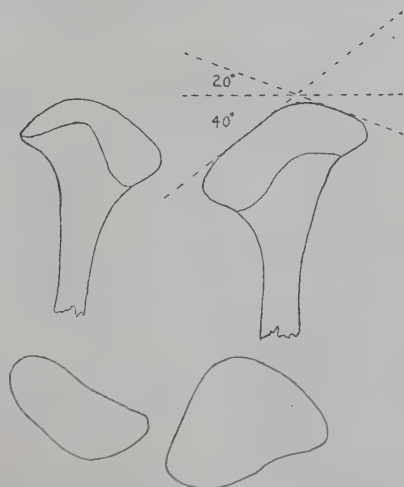
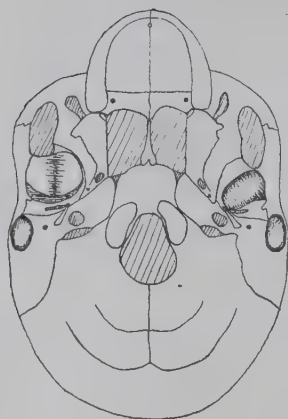
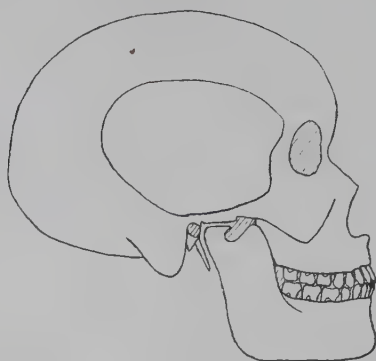


Fig. 1 Drawing of condyles of mandible. The upper figures show the condyles as seen from behind; the line below the upper part of the drawing gives the edge of the articulating surface. The lower figures show a view of the condyles from above. In each case the right hand figure represents a view of the pathological right side, showing its enlarged and altered articulating surface. The dotted lines show the approximate number of degrees of slope from the horizontal of each side of the joint surface.

surface running mesially from the ridge makes an angle of about forty degrees—being about twice as steep (fig. 1). The reason for this appears when we examine the planes in which the closing muscles act. The temporal muscle pulls practically vertically, not tending to dislocate the condyle either mesially or laterally. The masseter pulls laterally at an angle of twenty degrees from the vertical, tending to pull the condyle outward, the internal pterygoid tends to pull the condyle inward, as it acts at an angle



2



3

Fig. 2 Drawing of base of skull to show articulating surfaces for mandible. The broad oval area on the left of the figure is the pathological troughlike surface, articulating with the right condyle, shown in figure 1. The narrower oval of the opposite side of the skull is almost normal, although neither articulating surface extends as far back as the Glaserian fissure (shaded).

Fig. 3 Drawing of right side of skull. This shows the flattened articular surfaces of the pathological right jaw-joint, which allow the condyle to move forward and backward only in a straight line. The line of pull of the lower head of the external pterygoid muscle makes an angle of about five degrees with that of the joint surfaces, and is so directed as to pull the mandible against the skull during contraction.

of forty degrees to the other side of the sagittal plane. These lateral and mesial strains are exactly met, and in proper proportion, by the two sloping surfaces of the condyle as they are applied against the sides of the trough in which they run.

I further noticed that, although the surfaces of the condyle of an ordinary skull did not show these suggestive angles, if I placed an artificial meniscus above it—one molded in wax, fitting exactly that particular specimen—then the same significant slopes were seen in a transverse section of the condyle plus its meniscus.

While the study of this specimen perhaps adds little that is entirely new to our knowledge of the jaw-joint, it certainly gives very tangible proof of certain conditions, ordinarily not readily seen.

LABORATORY AND TECHNICAL MISCELLANY

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SIX FIGURES

AN ODORLESS DISSECTING ROOM

To attain this desirable end we make use of the ordinary chemicals, namely: glycerine, carbolic, alcohol, formaline, and find that it is largely a matter of quality and proportion. Since the impurities in the cruder carbolic acid cling to, if not penetrate, the living as well as the dead, we have avoided its use altogether and substituted a much better grade of acid with a melting point of 35 to 38° C. Moreover, since carbolic acid of 33 per cent strength usually crystallizes especially in the viscera of the cadavers and in this strength always is an obstacle to efficient work because of its anesthetic effects, we reduce the proportion of carbolic acid to about 10 per cent. This reduction in quantity also compensates considerably for the increased cost of the better grade of acid used, avoids the strong odors of the impurities and numbing of the fingers during dissection.

In addition to the carbolic we use from 2 to 2½ per cent of formaline, 20 per cent alcohol, and 20 per cent commercial glycerine. Since the formaline effectively fixes the material a larger quantity of alcohol than needed to get the carbolic to dissolve and the glycerine to flow seems wholly unnecessary. It evaporates quickly upon exposure carrying odors with it and also materially increases the cost. The large quantity of glycerine used also adds considerably to the cost, but I know of nothing that can replace it.

The total quantity of preservative used per cadaver should, to be sure, vary with the condition and size of the cadaver. From twelve to twenty liters is what we use. This keeps the cost of the preservative per body down to about two to three dollars, which is a sum sufficiently small to be within the reach of all laboratories and especially of those that can afford lead-lined storage tanks. Although we use from 12 to 20 liters of preservatives per cadaver we depend on gravity for pressure.

All our anatomical material is kept in a saturated atmosphere, in ordinary wooden plank tanks each tank requiring only about a gallon of methyl alcohol per year in spite of the dry climate. A very weak solution (½ per cent) of formaline will, of course, accomplish the same result as the methyl alcohol and for some time I have immersed some cadavers

in a very weak solution of formaline and glycerine in water for experiment. These and other contemplated changes in our method I hope to know more about in the near future. I am sure that anatomists are aware of the disadvantage attending the use of formaline and methyl alcohol but with proper care in the dissecting room drying can nevertheless easily be prevented while the material is under dissection. We avoid the use of vaseline because of its messiness.

It is very seldom that we use a body within less than a half a year after preservation. Most of our subjects are a year old. It is true that the use of the formaline makes dissection somewhat more arduous because of the consequent toughness of fasciae, but the compensations resulting from its use are so many and its disinfectant qualities so desirable that I have not cared to omit it. Streeter found as much as 6 per cent unobjectionable. Our material also loses much color but that is not a serious disadvantage. The attempt to retain the color by the use of potassium nitrate or by the later use of alcohol during dissection has not been of sufficient success in our experience to warrant their continuation. Although the quantities of carbolic and formaline used are relatively small both are present in sufficient amounts to assure thorough disinfection and the bogey of infections still paraded in some manuals has never been encountered in my experience. We keep no surgical dressings on hand, for unless infected otherwise, all cuts sustained in the dissecting room heal promptly.

I realize, to be sure, that the condition of the cadavers when received should determine how much and to a certain extent also what kinds of preservatives are to be used. Everyone has his preferences but from what I have experienced in several laboratories and seen and smelled in others, I cannot hesitate in expressing my preference for a method which enables an instructor to be in the dissecting room for a whole day without subsequently revealing the fact on the street or in a banquet hall. Nor need students who have not changed their garb become a nuisance even to those near to them. The proper use of a good hand lotion will also accomplish much in this respect.

In one laboratory with which I was connected it was always necessary in the spring of the year, to scrape off the mold every morning before beginning dissections. Since mold grows in the presence of strong carbolic and formaline and as far as I have experienced in all climates, proper mechanical protection against contamination seems the most efficient and the simplest means of avoiding it. I would not imply, however, that the nature of the preservatives bears no relation to the growth of mold, for I am convinced that the use of arsenic is for this reason alone very objectionable.

No doubt, someone has suggested our climate as our best friend and most efficient helper. I gladly admit its beneficence but the same results can and also have been accomplished elsewhere by similar methods.

As preservative to moisten the material while dissection is in progress we use essentially the lotion used by Professor Mall for years—decades. But we cover the cadavers and tables with white oilcloth. It prevents

evaporation very well, looks neat, is cheap, can be discarded as soon as soiled and is therefore far preferable to oiled muslin used perennially.

Anatomists need not be reminded that in an absolute sense the above caption is, to be sure, a euphemism. Yet visitors to our laboratory both from at home and abroad have used that expression and lest there be skeptics I shall add that a medical visitor from Berlin, for example, repeated the words 'Geruchlos' and 'Sonderbar' on going through the laboratory. Likewise, an eminent English physiologist who was compelled to see the laboratory literally on the run, ejaculated as he hastily went through the dissecting room, "Well, I declare! Quite-unlike-the-old-dissecting room! Smells sweet!" Other instances could be given, but enough, and this much not as a toot from our own horn but merely for the benefit of the skeptic.

Architecturally our laboratories are not ideal; but we as others have hopes. Although the ceilings of our laboratory are sixteen feet high there is no ventilation except by means of a few three-foot-square sashes placed between six to nine feet above the floor. Steam heat is used. In spite of these things our laboratory is practically odorless. We don't deoderize the cadavers simply because we don't know how but we do not add to the natural odor through the use of preservatives from which there is less escape.

THE STAINING OF ELASTIC TISSUES

While using the various elastic tissue stains on the fetal vessels I noticed that sections stained in a watery solution of orcein and mounted in glycerine always showed a much more brilliant stain. In fact fine elastic fibers, which for some reason were scarcely noticeable by other methods, stood out very distinctly by the use of this method.

MORDANTING WITH IODINE FOR MALLORY'S CONNECTIVE TISSUE STAIN

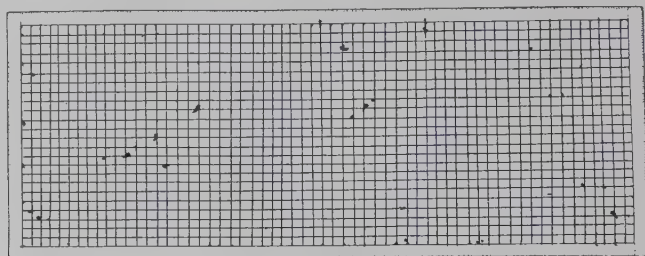
For staining the reticulum of lymph and hemal nodes by Mallory's method a special fixation as recommended by him is desirable. That this is preferable is admitted but it was found that by a preliminary mordanting of sections in a very weak iodine solution, before staining, almost any kind of fixation would yield a fair result with Mallory's stain. The advantage of this procedure lies, to be sure, only in the fact that it is an emergency measure which often enables one to use this special stain on material fixed by other methods than the preferred.

MESH DISSECTING TABLE TOPS

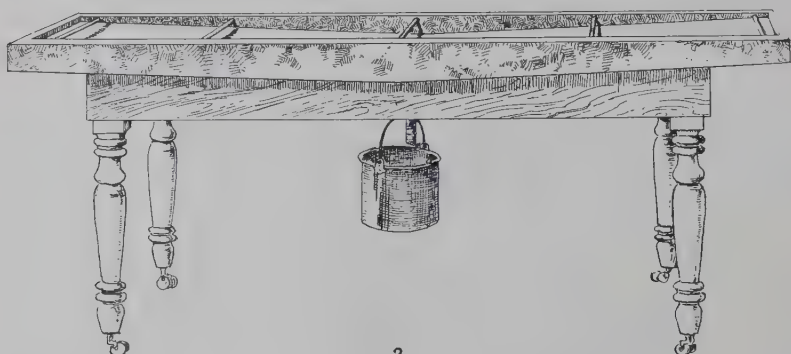
The hypostatic accumulation of the preservative and of the body fluids in some bodies and especially in connection with the use of certain methods of preservation suggested the use of a wire-mesh, false table-top. Such a table-top also prevents the accumulation of fat and the preservative which is applied externally during dissection on the

table and keeps the cloth wrapping from becoming soaked and unsightly. Moreover, it makes the use of water for cleansing the material easy and agreeable.

Figure 1 shows the wire mesh top with the zinc binding. We use No. 14, 2×2 galvanized iron wire mesh but would far prefer an aluminum mesh were it not so expensive and so flexible. Such a removable



1



2

Fig. 1 Mesh table top

Fig. 2 Dissecting table ready for mesh top

mesh top can be used on any kind of table for, as shown in figure 2, it is merely supported by removable rods. Since this mesh and the zinc table tops can quickly be cleaned with a hose when desired without removing the body, the accumulation of small pieces of material can be prevented easily and the tables kept clean throughout the progress of the dissection. The meshes are about one centimeter square.

If desired, a cork stopper can be placed in the drainage pipe or a valve can be put on it so as to permit preservative or water to stand on the table beneath the mesh. This effectively prevents drying even when a body is used for a long time for demonstration purposes.

MESH-BOTTOM AND FELT-SEALED* SPECIMEN BOXES

One of the disagreeable features usually connected with the study of dissected and injected preparations and frozen sections is the fact that it is customary to keep them in boxes or jars containing a solution of carbolic acid. Indeed, in some laboratories such specimens are kept wholly immersed in a weak (5 to 10 per cent) solution of crude carbolic acid although as a matter of fact crude carbolic will dissolve in water only to the extent of about 3 per cent. Hence it is necessary to roll up the sleeves sometimes as far as the elbow, in order to remove the specimens for study. When removed the specimens also run and drip with the crude carbolic solution. In order to obviate these objections we have placed false bottoms in the usual galvanized iron boxes and avoided the use of crude carbolic. These false bottoms are made exactly like the mesh table tops except that a lighter grade of wire can be used. They lie upon triangular rests made of narrow strips of galvanized sheet-iron. These rests are about two inches high and run across the width of the boxes at such intervals as may be necessary properly to support the specimens (fig. 3).

The mesh is covered with oilcloth or muslin, and a piece of muslin, or cheesecloth, long enough to pass completely around in the box is put with its midpoint on the true bottom beneath the triangular rests, the free ends of the cloth extending up each side of the box. A small amount of a weak preservative containing but little or no alcohol is then poured into each box and the free ends of the muslin put over the specimens which lie on the false bottom. Capillarity keeps the cloth moist and the specimens can be removed at any time without the possibility of unnecessary soiling. They are always moist but never wet, dripping or macerated. Sections of frozen infants kept in such boxes for a period of four years are still in excellent condition in spite of frequent handling.

In order to prevent evaporation these boxes are provided with a gutter for the flange of the cover, as shown in figure 3. This gutter is lined with felt one inch thick, which can be fastened with a shellac or paraffine paint and which seals the boxes so effectively that suction is very evident when the lids are lifted. If desired, the gutters can also be filled with glycerine but this has never been necessary even in our long dry summers.

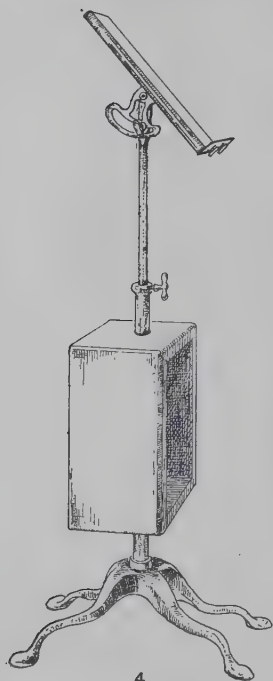
Coating the interior of the boxes with paraffine will delay corrosion from formaline-preserved material but in the course of years the boxes nevertheless get rather unsightly. Hence, I am at present arranging to have similar boxes made in white enamel. These will be permanent and far preferable esthetically.

DRAWING AND BOOK STANDS

The need of convenient and stable individual book and drawing stands has been met variously in different laboratories. Although individual stands require more space and often make a room look



3



4



5

Fig. 3 End view of specimen box; triangle supports and mesh bottom in place.

Fig. 4 Drawing and book stand. Fig. 5 Adjustable wire dissecting stool.

somewhat disorderly yet ever since my student days I have been so thoroughly convinced of their advantages that I have gone to some trouble regarding the matter.

The stand shown in figure 4 has been in use in our laboratory for over five years. It is exceedingly stable and not one stand so far has

required repairs. The heavy base has four rather than three legs, for stability. The legs are not provided with castors, for the same reason, but castors can be added in a moment, for holes are provided. The book box, which measures $12 \times 9\frac{1}{2} \times 6$ inches, is rotary on the stand. Hence the student can always easily reach the books. The oak drawing board, which cannot warp, is 12×6 inches in size. It is adjustable for slant and height and the rod supporting it can also be rotated. It is provided with a metal retaining edge which can also be adjusted for height, and which can be dropped to the level of the board.

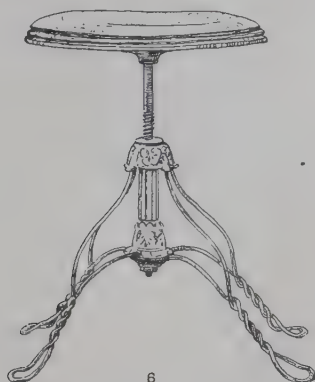


Fig. 6 Adjustable wire laboratory stool

The whole stand, which is easily dismantlable, is finished in white enamel except the metal supporting rods, which are nicked, and the drawing board, which is finished in dark oak. The metal retaining edge is antique copper. This stand, which has answered our needs completely, can be obtained from Frank S. Betz, Hammond, Indiana, at prices from five to six dollars each, varying somewhat with the size of the order.

STOOLS FOR DISSECTING ROOMS AND HISTOLOGICAL LABORATORIES

While looking about for a durable and comfortable stool for the dissecting room, over half a decade since, I had the good fortune to be referred to the Chicago Wire Chair Company. I have not become a stockholder since. This company was then making a stool which answered our purposes provided we could be supplied with a longer screw assuring a greater range of adjustment. Through the courtesy of the manager of the company this was easily accomplished and we have equipped all our laboratories with the stools represented in figures 5 and 6. The latter is a stock model and can be purchased almost

anywhere but the former is modified to suit our needs. It is adjustable for a height of 18 to 26 inches and can easily be provided with metal ring foot-rests placed higher up than the rectangular rodbraces between the legs, if desired.

These stools, which are practically indestructible, are not at all expensive. Only one stool was damaged in six years and the parts that wear out or may break can easily be replaced.

The metal parts are finished in antique copper, although other finishes can be obtained. Both stools can be obtained with or without backs. From an experience of six years I am inclined to prefer both without backs, since an occasional careless student uses the back to knock a stool over or to tip it back while sitting on it, thus eventually loosening the screws in the oak top. The tops or seats are so built as practically to preclude warping. The address of the makers is La Salle Avenue and Ontario Street, Chicago. The only justification for this note and the accompanying illustrations lies in the frequent comments and inquiries of visitors and their durability and very reasonable cost.

LETHAL CHAMBER

The essential thing in the construction of this chamber for killing small animals (dogs, cats, etc.) with illuminating gas, is a galvanized sheetiron false bottom which rests and slides on galvanized sheetiron right-angled strips attached to the walls of the chamber. In order to protect the zinc binding of the mesh bottom from soiling, an apron of wood, or preferably of sheetiron, is fastened to all sides of the box above the border of the mesh. Beneath the mesh bottom is a galvanized iron pan which is easily removable.

The animals are placed in the chamber, the front of which is provided with a well-fitting door, and the gas turned on gradually. If this is properly done dogs seldom utter a sound. When the animal is dead the removable mesh bottom and pan can be hosed off, thus keeping the chamber clean. Soiling of the animals is also made much less likely by the use of the mesh false bottom.

ANIMAL CAGES WITH HINGED BOTTOMS

In handling dogs it is often inconvenient to lean far into the cages in order to reach the retreating animal. To obviate this difficulty a slightly raised and inclined board floor two feet wide can be provided on the near or door end of the cage. The rest of the floor can be made of a galvanized iron mesh which is hinged near the wooden floor on a galvanized iron rod, so that the farther end of the mesh floor of the cage can be raised by means of a rope or wire from the door end. This compels the animal to come forward to the door and makes inspection and removal very easy for both animal and caretaker.

SECTIONAL PORTABLE LABORATORY CASES

It is often a great convenience to be able to move cases from one laboratory to another to meet some temporary need. Hence a special type of case with two sliding glass doors each in the base and top was designed. These cases can be built in one piece or the base and top can be built separately, and then fastened to each other by a few screws when placed in position.

Our cases are approximately 30 inches wide and 6 feet high, inside measure. The base is 2 feet deep but the top only 16 inches, leaving an offset of 8 inches which serves as a shelf. Since the doors are not hinged, anything standing on the offset need not be removed before the doors are opened and there is no danger of sweeping things off onto the floor when the doors are opened hastily. Directly beneath the offset are two drawers 5 inches deep, placed side by side. The central position of the drawers makes them very accessible. The size of the drawers and that of the unit itself can be made to suit personal preferences. The sliding doors can be provided with showease locks or they can be locked with a metal peg (a sawed off 20-penny spike) and a hasp and padlock. The peg is placed in a hole which passes through the overlapping sashes in the middle and the hasp is brought over it and locked in place.

DRAWING FROZEN SECTION

Few students of anatomy attain sufficient command over pen, pencil and brush to be able to make rapid satisfactory freehand drawings of frozen sections. Moreover, even if they had this command of drawing, the time consumed would be entirely too great when it is desired to make a considerable series of life-size drawings. We have obviated this difficulty by making rapid tracings on glass with India ink directly from the specimens, as is commonly done. These plates are then put on an inclined frame and illuminated from below by an electric light. By placing drawing paper over the ink tracing an accurate copy can quickly be made in pencil or ink and the details filled in from the specimen. The latter are handled on a wooden tray and are always turned over between two trays or a tray and a glass pane, to prevent damage. Pencil drawings can be made permanent in the customary way with shellac. The ease with which drawings can be made in this way encourages students to get as complete a series as possible for later reference, as well as for immediate use.

NEUTRAL STAINS AS APPLIED TO THE GRANULES OF THE PANCREATIC ISLET CELLS

W. B. MARTIN

From the Anatomical Laboratory of the Johns Hopkins Medical School

Through the investigations of Bensley and his pupils¹ we are aware that two types of cytoplasmic granules are present in the cells composing the islets of Langerhans in the pancreas of most mammals. These granules are distinct from those found in normal pancreatic parenchyma cells and this enables us to identify islet tissue. Bensley's valuable contribution rests on the application of a neutral dye (e.g., Reinke's neutral gentian) to pancreatic tissue which has been fixed in a particular way. At the suggestion of Dr. H. M. Evans, therefore, this work was taken up with the view of determining the best method of preparation of the neutral dyes, the concentration of the staining solution necessary for the best results and further to investigate certain of the dyes allied to gentian violet and orange G, in the hope of obtaining a neutral stain more efficient than Reinke's neutral gentian.

Gentian violet is a mixture of two dyes of the triphenylmethane series, hexamethylpararosanine and pentamethylpararosanine. Therefore, when gentian violet is combined with orange G the resulting neutral stain is also a mixture of two dyes and it is this mixture that is known as neutral gentian. As the two components of gentian violet differ somewhat in their staining properties and as the relative proportion of these constituents vary in different samples of gentian violet on the market it would seem advantageous to substitute the pure hexa or penta compound for the mixture. This at once suggests the substitution of other dyes of the triphenylmethane group on the basic side of the reaction and also the replacement of orange G by other acid dyes of the azo series. This idea has been carried out and a number of neutral stains prepared. These dyes have been applied to pancreatic tissue fixed by the Bensley method. The result of the study of these neutral dyes is set forth below with a brief description of each dye.

The acid dyes and basic dyes combine in molecular proportion. In some cases the ratio is a simple one of 1 : 1 and in other cases the ratio may be 1 : 2 or 1 : 4. For example, ethyl violet combines with ponceau

¹ M. A. Lane, The cytological characters of the areas of Langerhans. *Am. Jour. Anat.*, vol. 7, 1907; R. R. Bensley, Studies on the pancreas of the guinea-pig. *Am. Jour. Anat.*, vol. 12, 1912.

4 G B in the ratio of 1 : 1, with orange G in the ratio of 2 : 1 and with trypan blue in the ratio of 4 : 1.

The method of preparation of the neutral dye is the same in each case. A concentrated aqueous solution of the acid stain is added to a similar solution of the basic substance. This should be done slowly and the mixture stirred thoroughly. The neutral point may be determined in the following manner: After each addition of the acid substance the mixture is stirred and a drop is taken on a glass rod and placed on a piece of ordinary filter paper. The neutralized portion of the mixture, being in the form of a precipitate, settles at once on the paper, while the liquid portion containing the unneutralized stain spreads in a circle around the deposit. By the color of the outer ring can be determined whether the solution contains an excess of the acid or the basic dye, and by the change in the degree of colorization one can readily perceive the approach of the neutral point. When this is reached the outer ring is entirely colorless. The end point is thus made as exact as in any other chemical reaction and the filtrate in such a case is either clear or only slightly colored. One thus avoids an excess of either stain and the residue is practically free from either of its single constituents. This is of practical importance, for the failure to free the neutral stain of one of its components may account for some of the difficulties that have been encountered in the use of neutral gentian. The residue obtained above is filtered, washed with distilled water and allowed to dry either in the air or in an oven at a low temperature.

In order to determine the concentration of the staining solution necessary to obtain the best results, solutions of varying strength were used, the staining time remaining fixed. A solution of known strength was made up in absolute alcohol and this was kept as a stock solution. From the stock, staining solutions in 20 per cent alcohol ranging in concentration from one of 8 mgm. of the solid dye in 50 cc. of alcohol to one of 0.25 mgm. of the dye in 50 cc. alcohol were prepared. The strength of these various staining solutions is given in table 1. Positive or negative results are indicated by the corresponding mathematical signs. To obtain the best results fairly dilute solutions should be used. A solution of neutral ethyl violet orange G containing approximately 2 mgm. of the crystal dye to 50 cc. of 20 per cent alcohol is satisfactory. A staining solution of neutral azo fuchsin should contain 0.5 to 1.0 mgm. of dye to the same amount of alcohol.

When brought together in molecular proportions these dyes react with the precipitation of a neutral dye. On drying, this is a dark green powder giving a deep purple violet color in alcohol:

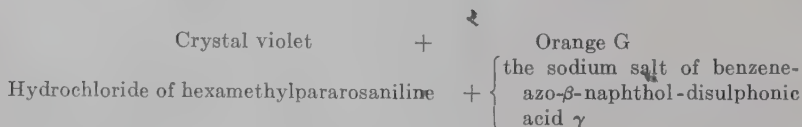


TABLE 1

Showing relative staining power of different neutral dyes. The numbers refer to Schultz's Farbstofftabellen, 1914

Milligrams of neutral dye added to 50 cc. of 20% alcohol for staining solution	8	4	2	1	0.5	0.25
Orange G(38) (A) + gentian violet	+	-				
+ penta methyl violet (515) (C.J.)	+	-	-			
+ hexa methyl violet (516) (C.J.)	+	+	+	-		
+ crystal violet (517) (B)	+	+	-	-		
+ ethyl violet (518) (B)	+	+	+	+	-	
+ victoria blue B (559) (B)	+	+	+	+	+	-
<i>blue</i>						
Ethyl violet + azo eosin (94) (B γ)	-	-				
+ brilliant croceine 3B (227) (B γ)	+	-	-			
+ croceine orange G* (B γ)	+	+	+	-		
+ Ponceau 4 G. B. (37) (A)	+	+	-			
+ diphenyl brown (347) (Sch)	+	+	+	-		
+ diamine brown (344) (Sch)	+	+	+	+	+	-
+ azo fuchsine G (146) (B γ)	+	+	+	+	+	+
+ trypan blue (391) (C)	+	+	+	+	+	+
+ heliotrope B** (Sch)	-	-	-			

* From Fabrenfabrik of Elberfeld Company. Although given by Schultz in his last edition (V) as identical with ponceau 4 G. B. the dye is undoubtedly different.

** Not given in the last edition of Schultz under this name.

Methyl violet

+

Orange G

Hydrochloride of pentamethylpararosanine + { sodium salt of benzene-azo-
 β -naphthol-disulphonic acid γ

Hexa methyl violet, which is given in the table, has the same composition as crystal violet but is a purer product and gives slightly better results. Both of these dyes react with orange G in the same manner, giving a coarsely crystalline neutral dye of a green color and with a fine metallic luster. Sections of pancreatic tissue stained in any one of the above dyes present much the same picture, though the hexa methyl violet is decidedly superior to the penta methyl violet. In each case the zymogen granules of the parenchyma are stained a vivid heliotrope on a light orange background. The nuclei of all the cells are blue or violet, and in the islets of Langerhans the granules in A and B cells of Lane are differentially stained in the same manner as when stained in neutral gentian. This is to be expected, as gentian violet is a mixture of methyl violet and crystal violet and in staining power lies intermediate between the two:

Ethyl violet

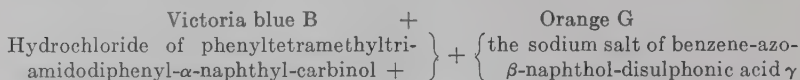
+

Orange G

Hydrochloride of hexaethylpararosanine + { the sodium salt of benzene-azo-
 β -naphthol-disulphonic acid γ

Triphenyl

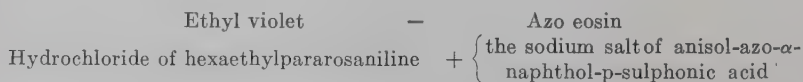
Ethyl violet and orange G react in the proportion of 2 : 1 in the usual way, giving rise to a neutral stain. This forms lustrous green crystals having a slightly bronze cast and gives a violet solution in alcohol. This stain is much superior to any of the above. While the general picture is the same, it is much more intense in its action and the three types of granules are more sharply differentiated. It has the advantage also of resisting the action of the dehydrating and differentiating agents better:



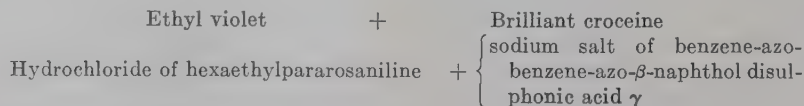
The neutral dye is obtained in the same way and forms lustrous crystals of a deep brownish-red color. The alcoholic solution is blue without the red cast of the stains so far mentioned.

Sections stained in this dye present a somewhat different picture. The zymogen granules are stained blue on a pale yellow background. The granules in the islet cells are stained a more intense blue and retain their color when differentiated from a dilute solution longer than the zymogen granules. The nuclei of all the cells are stained a beautiful blue green, the nucleolus and chromatin network standing out clearly against the rest of the nucleus. This dye will stain in high dilutions, but on account of the deficiency in color contrast between the granules and protoplasm is not as desirable a stain as the neutral ethyl violet.

The series of neutral stains so far given have been combinations of orange G with various basic dyes of the triphenylmethane series. Of these ethyl violet is the most valuable. In the following experiments this dye has been joined to a number of acid dyes of the azo series and the staining power of the resulting compound studied:



The neutral stain crystallizes, forming bronze green crystals, and gives a deep violet red solution in alcohol. It is, however, lacking in staining power and in concentration as high as 32 mgm. of dye to 50 cc. of 20 per cent alcohol stains the zymogen granules very faintly and the nuclei not at all:



Two parts of ethyl violet combine with one part of brilliant croceine. The neutral stain is a lustrous crystalline substance of a fine green

color forming a violet red solution in alcohol. The protoplasm of the cells stains a pale pink while the zymogen granules take a slightly darker shade. The granules in the islet cells are stained faintly and the two types can be made out but the differentiation is poor:

Ethyl violet + Croceine orange G

Fine bronze crystalline substance giving a violet red solution in alcohol. Sections stained in this dye are a bright yellow. The zymogen granules are a reddish brown. The granules of the islet cells of one type are stained a deep blue while the other type takes the same yellow color as the background. In higher dilutions the zymogen granules still take the stain but the islet granules are not stained. The nuclei are shown fairly well:

Ethyl violet + Ponceau 4 G. B.
Hydrochloride of hexaethylpararosaniline + { sodium salt of benzene-azo- β -
naphthol- β -sulphonic acid

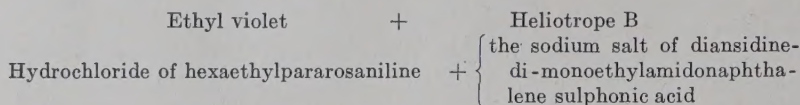
These dyes combine in the proportion of 1 : 1, forming a gummy residue which crystallizes out on standing and gives a deep red solution in alcohol. The staining action of this dye is very much like that of the neutral ethyl violet orange G compound except that it is dissolved out much more rapidly by differentiating agents and on this account is entirely unsatisfactory:

Ethyl violet + Diphenyl brown
Hydrochloride of hexaethylpararosaniline + { sodium salt of
benzidin { salicylic acid
monomethylami-
donaphthol-sul-
phonic acid

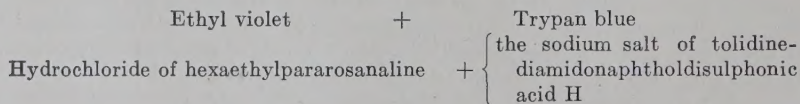
Ethyl violet and diphenyl brown combine in the ratio 2 : 1 to form a neutral stain. This is a dull black amorphous powder giving a violet alcoholic solution. The zymogen granules appear heliotrope on an orange background. The granules in the islet cells take the stain but they are not sharply differentiated:

Ethyl violet + Diamine brown
Hydrochloride of hexaethylpararosaniline + { the sodium salt of
benzidin { salicylic acid
amidonaphtholsul-
phonic acid

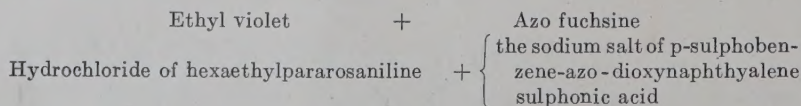
Ethyl violet combines with diamine brown in the same proportion as with diphenyl brown to form a neutral stain. This is a dark brown powder giving a violet red solution in alcohol. Stained sections show dark red zymogen granules on a pink background. The nuclei stain exceedingly well. The granules however in the islet cells are not well differentiated and take a shade of red not very different from the background. The general picture resembles that seen in sections stained in neutral azo fuchsin except for the character of the islet granules.



This is a dark brown amorphous powder giving a deep violet alcoholic solution. Zymogen granules are stained a light heliotrope and the protoplasm a faint pink. The granules in the islet are not well stained. This was not tried in concentrations higher than 8 mgm. of stain to 50 cc. of 20 per cent alcohol and since it offers a fairly good color contrast it might be useful in higher concentrations.



Four parts of ethyl violet are required to neutralize one part of trypan blue. The neutral dye is a deep green crystalline substance. The alcoholic solution is blue with a slight violet tint. This is a very intense stain and resists the action of alcohol and acetone. The zymogen granules are stained a deep purple blue against a light blue background. The nuclei stain fairly well. The differentiation of the granules in the islet cells is however poor. Both types appear to take the stain but the color contrast throughout is not sharp enough:



Ethyl violet and azo fuchsin combine in the ratio of 2:1. The dry neutral dye prepared in the same way as the above is a fine crystalline powder of a green color which gives a violet red solution in alcohol. Pancreatic tissue stained in this dye presents a very brilliant picture. The zymogen granules are a deep purple on a light pink background. The nuclei in all the cells are stained fairly well, the chromatin material being red. The two types of granules in the islet cells are stained differentially, one type taking a violet stain and the other a distinct red. This is a very intense stain and may be used in high dilutions.

It is resistant to the action of acetone and alcohol thus making possible a more careful differentiation than with any of the other stains used. Sections stained in it have little tendency to fade and preparations made nearly a year ago are as brilliant as when first made.

From a consideration of the group of dyes just described it is evident that two of them stand out as distinctly superior to any of the others as a stain for pancreatic tissue. These are the compounds formed by the union of ethyl violet with orange G and with azo fuchsine. These are both powerful dyes, staining cell granules very intensely. The color contrast between the different types of granules and between the granules and the cell protoplasm is very sharp. They are efficient in high dilutions and gross precipitation of stain on the tissue is avoided. Finally it may be said the granules and the nuclear chromatin retain these stains well in the presence of acetone and absolute alcohol, thus rendering differentiation easy.

On referring to the table given above it is seen that the neutral stains formed by combining orange G with different basic dyes vary in staining strength and that as the basic substance used becomes more complexed the staining power of the neutral dye increases. Thus, the hexamethyl violet gives a more efficient stain than the penta methyl violet, the hexa ethyl compound surpasses the hexa methyl combinations while victoria blue joined to orange G gives a neutral dye that will stain efficiently in higher dilutions than any of the others.

The dyes tested in the above study were not secured through dealers but in each instance from the firm concerned in its manufacture. I am indebted to Dr. Evans, who placed his collection at my disposal, and we wish to thank the following houses for coöperation both in the supply of dye samples and in the confirmation of the precise chemical make-up of the dyes used: Farbenfabrike of Elberfeld Company; the Badische Company; the Berline Aniline Works; Kalle and Company; Leopold Cassella & Co.; Carl Jäger; and Schoellkoff, Hartford & Hanna Co.

INCREASE IN PRICE OF JOURNALS

In order to extend and improve the journals published by The Wistar Institute, a Finance Committee, consisting of editors representing each journal, was appointed on December 30th, 1913, to consider the methods of accomplishing this object. The sudden outbreak of European misfortunes interfered seriously with the plans of this committee. It was finally decided, at a meeting held December 28th, 1914, in St. Louis, Mo., that for the present an increase in the price of these periodicals would not be unfavorably received, and that this increase would meet the needs of the journals until some more favorable provision could be made.

This increase brings the price of these journals up to an amount more nearly equal to the cost of similar European publications and is in no sense an excessive charge.

The journals affected are as follows:

THE AMERICAN JOURNAL OF ANATOMY, beginning with
Vol. 18, price per volume, \$7.50; foreign, \$8.00.

THE ANATOMICAL RECORD, beginning with Vol. 9, price
per volume, \$5.00; foreign, \$5.50.

THE JOURNAL OF COMPARATIVE NEUROLOGY, begin-
ning with Vol. 25, price per volume, \$7.50; foreign, \$8.00.

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